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Grain skinning in malting barley: The influence of husk adhesion on grain and malt quality

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Declaration

This is to certify that that the work contained within has been composed by me and is entirely my own work. No part of this thesis has been submitted for any other degree or professional qualification.

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Glossary and Abbreviations

ABA - Abscisic Acid. Plant hormone, it is responsible for preventing grain germination and maintaining dormancy

AHDB - Agriculture and Horticulture Development Board (AHDB). Levy board which represents farmers, growers and others in the supply chain.

AdjHWE – Adjusted Hot Water Extract. Calculation developed in this thesis to account for the skinning severity of the bulk grains

ANOVA – Analysis of Variance. Statistical test, which estimates the variation between and within groups.

ASAE – The American Society of Agricultural Engineers (ASAE). An international professional society devoted to agricultural and biological engineering.

ERF – Ethylene Response Factor. Part of the ethylene signalling pathway.

GA - Gibberellic acid. Plant hormone responsible for initiating plant germination and growth

GS – Growth stage. It is a guide, which helps in precise determination of the stage of plant development for precise application of treatments

Husk-loss type – refers to the missing part of the husk, in this work two types were examined “missing lemma” (lemma part of the husk was detached, palea was intact) and “huskless” (both lemma and palea detached)

HWE – Hot water Extract (L^o/kg). It is a measure of malt modification; it uses density of the sample to estimate the sugars dissolved in the extract

IoB – Institute of Brewing. It is an industry trade association for brewers and distillers, currently called Institute of Brewing and Distilling (IBD). IoB compiled standard methods of malt quality assessment, which have been incorporated into European Brewing Convention Methods.

MC – moisture content. The measure of the water present in the grains, and can be presented as % dry basis (db) or wet basis (wb)

Modification - the extent of cell wall breakdown in barley endosperm. Well modified grains have hydrolysed cell wall but not starch; overmodified grains have hydrolysed cell walls and some starch in the endosperm

SEM – Scanning Electron Microscopy

Skinning – undesirable condition of malting barley grains, in which grains lose more than 1/5 of the husk

Skinning mechanism – describes the grain tissue affected in skinning. ‘Cementing layer’ is weakening of adhesion along cementing layer, ‘parenchyma cells’ is the breakage of parenchyma cells of the husk.

Skinning severity – in this work it describes the severity of the husk-loss defect in the bulk of grains, and often described as a percentage of grains within the bulk, which are skinned

SRUC – Scotland's Rural College.

SW – Specific weight is the measure used in cereal evaluation. It is a weight of the grains in a specified volume

TCW – Thousand corn weight. Weight of one thousand grains, used in cereal evaluation. Higher weight indicates big, plump grains

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Abstract

Barley (*Hordeum vulgare* L.) is a crop of prime importance to the malting, brewing and distilling sectors. Modern varieties are susceptible to a defect called "skinning", a condition in which the adhesion of the husk to the caryopsis has been weakened, and partial or complete loss of the husk occurs. Malting of skinned grains is inefficient and results in production of poor quality malt and financial losses to the farmers and maltsters. Although the malting industry recognises that grain skinning causes inefficiencies and loss of production, to date there has been no quantitative assessment of these effects. Furthermore, there is little understanding of how the condition of harvested grain (e.g. moisture content) influences the occurrence of skinning, and thus its potential value in the malting process. The overall aim of this research was to investigate how grain condition at harvest influences the quality of husk adhesion and how skinning severity and husk-loss type impacts the malting process and quality of the malt produced.

This thesis details an investigation into the effects of moisture content (MC) to determine whether grain or tissue moisture content affects grain skinning. In the three varieties examined in the field, MCs were between 22% and 45% and skinning was positively correlated with MC; in the four varieties examined in the glasshouse, MCs were between 11% and 25% and skinning was negatively correlated with MC. Light microscopy examination of the grains has confirmed that two distinctly different mechanisms of skinning: in the glasshouse, parenchyma cells were damaged

whereas in the field tissue has separated along the cementing layer, without causing cell damage.

Micromalting studies were conducted on two varieties (Concerto and Chronicle), with four skinning severities: intact (0% skinned), mild (16% skinned), skinned (50% skinned) and severe (90% skinned), to examine the effect of skinning severity on the quality of the malt produced. Quality was determined by measuring friability and homogeneity, hot water extract (HWE) and α-amylase content. Under- and over-modification of the samples was also investigated at a single grain level, using the Carlsberg Malt Modification Method, thin layer chromatography and scanning electron microscopy. Hot water extract increased with the increase in severity of skinning, however when it was adjusted to account for lost husk biomass HWE then decreased with skinning severity. Friability, homogeneity and the α-amylase content also decreased with higher skinning severity. Detailed investigation into modification indicated that malt quality declines with increase in skinning due to a large proportion of undermodified grains in skinned malt. No signs of overmodification were detected.

Lastly, two most common husk-loss types in the bulk (missing lemma and huskless) were investigated and compared to grain with attached husk to determine if the type of husk damage is of importance during malting. The difference in germination rate and vigour, production of α-amylase and the rate of water uptake were investigated. Huskless grains had significantly lower levels of germination rate

and α-amylase production, compared to the grains with lemma missing and unbroken husks.

The main findings from this project are that grain skinning reduces malting efficiency and the quality of the malt produced due to undermodification of the grains and lack of homogeneity in the malted bulk. For the farmer, reduction in the skinning risk could be achieved through careful observation of the moisture during harvest. Maltsters would be able to achieve improved homogeneity and efficiency through careful examination of the bulks for the types of skinning and alterations in the malting regimes employed. On the methods currently used in micro-malting tests and variety evaluation, it is evident that malting of samples with high levels of skinned grain will give a HWE compared to samples with intact grain. Therefore, a corrected measure of HWE is required to take into account the proportion of skinned grain, or mass of husk-loss, in order to determine the true malting efficiency of the sample, or variety.

Lay Summary

Skinning is an undesirable grain condition in modern malting barley varieties, in which a partial or complete loss of husk occurs. There are many factors influencing the severity of skinning including genetics, growing environment and grain handling at harvest and during malting. This condition causes inefficiencies and losses in the whole barley supply chain: farmers lose premiums for poor quality grains; maltsters have issues with modification and homogeneity of the final product, and brewers and distillers buy poorer quality malt with higher prices. The aims of this project were to investigate how grain condition at harvest influences skinning and subsequently how different severity of skinning and husk-loss type affects the efficiency of malting. An important part of the malting analysis was to establish whether overmodification or undermodification played a role in the lack of efficiency.

In the first part of the project we looked at the final stages of the barley ripening, just before harvest, and effects of moisture associated with growth stage and with the environment (rainfall) on the severity of skinning through a series of glasshouse and field experiments. Moisture content associated with earlier developmental stage of the plant, where barley was harvested just before it was fully dried, had a protective effect on the grains. In contrast moisture content associated with the environment had the effect of worsening the severity of skinning. Light microscopy on samples from both experiments showed that skinning in each of those cases occurred at a different anatomical layer of the barley grain.

The hypothesis that skinning negatively influences the efficiency of malting process and impacts the quality of the final malt produced was investigated in the second part of the project. In the micromalting experiments two barley varieties were divided into four skinning categories ('intact', 'mild', 'skinned' and 'severe') based on the proportion of skinned grains in the bulk and quality of the malt produced was assessed using standard industry methods (friability, homogeneity, hot water extract and α-amylase activity). The main finding was that hot water extract increased with the increase in skinning severity, however when the lost husk biomass was taken into account in the calculations, the pattern was reversed and the least extract was recovered from malt with severe skinning. Other measures of malt quality including friability, homogeneity and α-amylase content confirmed that malt containing a very large proportion of skinned grains (~90%) did not modify as well as other categories. These results highlight couple of issues: skinning is affecting the efficiency of malting and use of HWE should be carefully considered, especially when the barley samples contain skinned grains.

The last aspects of experimental work of this project focused on investigating the effects of skinning on modification at a single grain level, and whether undermodification or overmodification are significant factors influencing the efficiency of malting and the quality of final product. Carlsberg modification method, analysis of the sugar composition of the extract using thin layer chromatography and scanning electron microscopy of malt reinforced the findings from the previous chapters, that skinning causes inefficiencies and lack of homogeneity in malt

produced from skinned samples and there were evidence of undermodification but no overmodification was detected.

In conclusion, skinning effects the quality of barley grains which in turn has an effect on the efficiency of malting and modification. Increased hot water extract in samples with skinned grains suggested better malting performance, but when carefully analysed this malt was undermodified, inhomogeneous and lack of husk masked the decrease in extract recovered.

Chapter 1. General introduction. Barley grain, skinning and malting

1.1 Background

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world. It is used in the production of food, animal feed and for malting. In the UK, malt is primarily used in the brewing and distilling industries, and in much smaller proportions in the production of confectionery and soft drinks. The quality of malting barley grains is of prime importance for maltsters, brewers and distillers, in order to maintain processing efficiency and product quality. Maintaining grain quality is supported by the development of new varieties through plant breeding and evaluation systems. During the evaluation, candidate varieties undergo rigorous testing to fulfil grain quality and processing criteria in order to be accepted onto cereal recommended lists such as the AHDB Recommended Lists for Cereals and Oilseeds (AHDB, 2019). Throughout their testing, candidate malting varieties are evaluated for a wide range of grain and malt characteristics. Yield is of primary importance when selecting a new variety, and only when it has outperformed the old varieties on yield will the candidate variety be considered for acceptance onto the list. In addition to being on the AHDB Recommended Lists, the quality of each barley batch destined for malting is tested at intake. Key barley grain criteria include

germination energy, grain size, moisture and nitrogen content. Good husk adhesion is one of the quality criteria that is becoming increasingly important; grains with poor adhesion lose a significant proportion of husk – a condition known as “skinning”. In recent years, grain skinning has become of increasing concern for the whole malting sector. Evidence suggests that modern varieties, with high malting quality, are expressing higher levels of husk detachment and loss (Brennan et al., 2017b). Grains of substandard quality could be offered a reduced premium, or bulks of grains may be rejected by maltsters and sold as feed barley, causing financial loss to the farmer. Such rigorous control of barley quality is necessary as malting poor quality barley results in reduced outputs and problems during processing of the malt in the brewery or distillery.

The occurrence of grain skinning in barley is now considered by the malting sector as one of the most important aspects of barley quality to address. Recent research has identified some of the factors that influence the quality of husk adhesion and cause skinning, including biological processes during grain development (Hoad et al., 2016; Brennan et al., 2017b), genetic influences (Brennan et al., 2019) and agronomic or environmental factors (Hoad et al., 2016; Froment & South, 2003). These recent findings may help to select and grow new varieties with improved husk quality. However, the impact of poor husk adhesion is still not fully understood, especially the effects which different levels of skinning and types of husk-loss have on the malting process and malt quality. The malting sector accepts that skinning results in reduced efficiency and loss of malt production, and these effects have been reported previously by Meredith (1959). However, that research was published 60

years ago, and no other research, especially in the context of modern varieties, has quantified the effects of poor husk adhesion on malt quality. One of the aims of this thesis was to understand whether variables such as environment and variety influence the quality of husk adhesion and the effects of skinning on malting quality. This could be used in informing the farmers of the best time and conditions at harvest and maltsters on how to process skinned barley to increase efficiency. Barley grain anatomy and quality, grain skinning and the malting process are presented in detail in this chapter.

1.2 Barley

1.2.1 Barley grain anatomy

Barley grain anatomy and details of layers surrounding the starchy endosperm are presented in Figure 1.1. During development of the barley grain, the dorsal lemma and ventral palea (which in mature grain comprise the outer husk) protect the flowering parts (the carpel, stamens, anthers and filaments), and then after flowering protects the developing caryopsis (the barley fruit), the outer tissues of which are the seed coat layers (testa) and the fruit coat (pericarp).

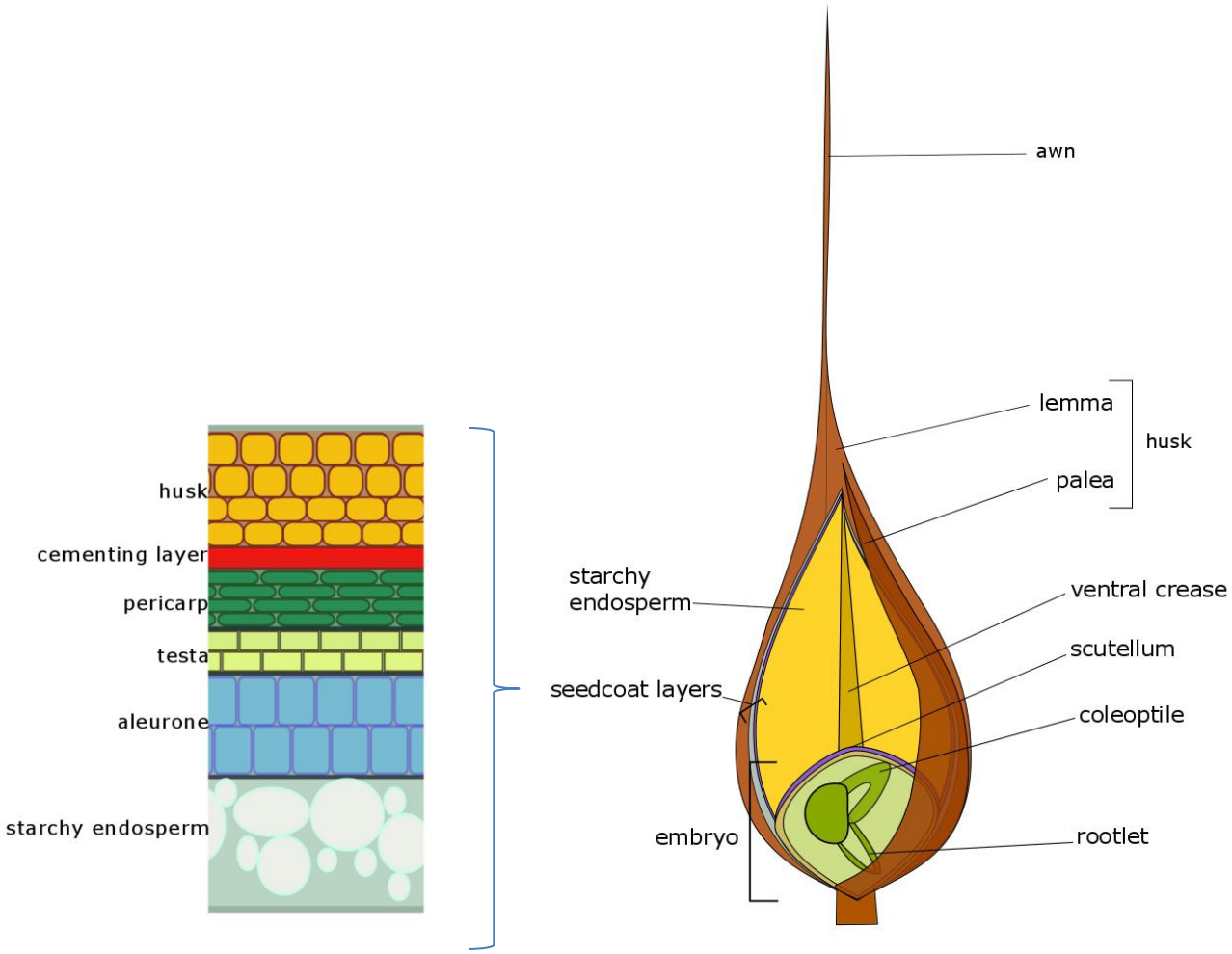


Figure 1.1. Anatomy of the barley grain and the layers surrounding the starchy endosperm.

The testa is a layer of cells, which are dead in mature grains and it is made up of a nucellar layer and a waxy cuticle, separated by the remnants of cell walls. It provides a protective layer to grains and it is the testa that contributes the most to protecting the living tissue of the endosperm from the environment. An excellent example of its strength and impermeability is that during decortication of grains with 50% sulphuric acid, where the husk and pericarp separate from the testa, the testa remains intact (Briggs, 1998). The pericarp is dry at maturity and in most cereals it consists mostly of large empty cells by this time. During development and maturation of the grains it contains chloroplasts and accumulates small quantities of starch, which toward the end of maturation disappear completely (Evers et al., 1999). The husk and pericarp provide protection to the living tissues of the caryopsis from mechanical damage and diffusion of gasses, water and solutes (Briggs, 1998). The aleurone layer is a living tissue, usually three cells thick, and it is a part of the endosperm, although its function is very different to the starchy endosperm. This tissue plays a crucial role in enzyme production and release during germination, triggered by the diffusion of gibberellic acid (GA) (Clutterbuck & Briggs, 1973; Palmer, 1972b). It also stores a small proportion of carbohydrates, including sucrose, which is used as an early source of energy for the embryo, before carbohydrates from starch are hydrolysed (Henry, 1988; Briggs, 1998).

Unlike other cereal grasses, towards the final stages of grain development the barley husk strongly adheres to the kernel (caryopsis). The husk constitutes between 10% to 17% of the grain weight and it does not contain any starch or sugars that contribute to the malt extract values (Evers et al., 1999). Adhesion of the pericarp to

the husk occurs usually around 10 days post anthesis (Harlan, 1920; Gaines et al., 1985). The adhesion is attributed to a cementing layer, which is waxy and cuticular in origin (Brennan et al., 2019, 2017a), and is produced by the pericarp. The composition of the cementing layer affects the quality of adhesion, and it is also likely influenced by the environmental factors during its development (Brennan et al., 2017a, 2019).

1.2.2 Mechanism and control of adhesion

The development of the cementing layer, which is crucial for husk to caryopsis adhesion, is controlled by the *nud* gene – a single recessive gene located on chromosome 7H (Franckowiak & Konishi, 1997). Through positional cloning, a laboratory technique used to identify a mutation underlying the pathological development of diseases, it was determined that this ethylene response factor (ERF) transcription factor controls the naked/hulled characteristic of the grain. This ERF has homology to an Arabidopsis WIN1/SHN1 transcription factor responsible for the synthesis of biolipids (Taketa et al., 2008). Duan et al. (2015) used an RNA-seq technique to identify differentially expressed genes in two (covered and naked) barley varieties. In that study, the *nud* gene was highly expressed in covered barley, with trace expression in the naked variety. The majority of the cuticle-related differentially expressed genes were down regulated in the covered barley. Some barleys with mutations in those genes described in Duan et al. (2015) result in increased permeability of the cuticles, including mutations of the *atwbc11* and *bdg* genes (Luo et al., 2007; Jakobson et al., 2016). Highly permeable cuticles are more

susceptible to organ fusion, which is a similar phenotype to husk adhesion in barley; this similarity has also been observed by Taketa et al. (2008) and Brennan et al. (2019).

Skinning is a type of physical damage which is of importance in malting barley grains, where partial or complete loss of the husk occurs. In the literature, the value of 20% of the area of the husk-loss is usually the cut-off point between intact and skinned grains (Brennan et al., 2017b, 2017a). A small proportion of husk missing at the distal end is usually a result of removal of the awn, and it is not considered skinning. Representative images of different types of skinning are shown in Figure 1.2.

Figure 1.2. Intact and skinned grains, with various proportions of missing husk.

Plant cuticles cover surfaces of all the plant organs and are composed mostly of long-chain hydrocarbons including alkanes, alcohols, aldehydes, ketones and esters (Fich et al., 2016; Yeats & Rose, 2013). The composition of plant cuticles determine their physical properties such as permeability (Shepherd & Griffiths, 2006), and as reviewed Bird & Gray (2003), highly permeable cuticles are more

susceptible to organ fusion. As the composition of plant cuticles are known to influence their physical properties, it may be that differences in the composition of the cementing layer among barley varieties and growing environments could be responsible for the quality of the adhesion (Brennan et al., 2016, 2017a, 2019). Organ fusion of plant tissues has a similar phenotype to husk adhesion. One of many functions of plant cuticles is prevention of organ fusion during development (Nawrath, 2006). Mutations in genes controlling cuticle production can result in increased permeability and organ fusion in *Arabidopsis thaliana* (Taketa et al., 2008; Takahashi et al., 2010). Additionally, evidence suggests that although the cementing layer is important, the structure of the husk (including size and thickness of the husk cells) could also play a role (Olkku et al., 2005).

1.2.3 Effect of the environment and grain handling on skinning

Skinning severity and susceptibility is dependent on a number of risk factors and their interactions. Genotype, including a functional *nud* transcription factor, determines the production of the cementing layer. However the differences in composition of this layer could be responsible for the varietal differences in the quality of adhesion and therefore skinning susceptibility, and it is not yet known if the NUD transcription factor controls quality of adhesion (Brennan et al., 2019, 2017a). Variety choice is very important, some varieties are more susceptible to skinning than others, even when the growing conditions are the same (Psota et al., 2011; Brennan et al., 2017b). However growing season and environments are just as crucial (Froment & South,

2003). Also, there are some modern cultivars which are less susceptible to skinning, but when the environmental factors, including moisture, are unfavourable they are prone to husk-loss (Aidun et al., 1990; Brennan et al., 2017b). Skinning has been much more prevalent in Scotland in recent harvests, especially those where intermittent cold and wet conditions have occurred during grain filling, or during the harvest period. This was observed previously in the field trials by Froment & South (2003), who suggests that rainfall (misting treatments) increased skinning. A similar result was obtained in more controlled misting treatment in a glasshouse, where seven varieties of commercial importance were chosen to test whether misting during grain filling and ripening significantly increased the skinning severity compared to un-misted controls (Brennan et al., 2017b).

Grain harvesting and post-harvest handling, including downstream processing, are critical phases during which partial detachment or complete loss of the husk can occur. Starting at harvest, improper settings of the combine harvester result in increased skinning. Vogel & Widdifield (1949) analysed samples from combine harvesters and showed that those with high speed lost higher proportions of husk. Damage caused by the combine harvester reduces the germinative potential of the grains (Mitchell et al., 1958). The malting process subjects grains to further physical stress as it involves mechanical agitation and transport, where grains can become damaged (Olkku et al., 2005). Conditions which are too dry are not ideal either as some evidence suggests it causes the husk to become brittle and flake off from the grains resulting in large quantities of dust being produced and a greater need for extraction during brewing/distilling (Olkku et al., 2005). Skinning is to a great

extent a condition with multiple causes and interaction of many factors that contribute to the weakening of caryopsis to husk adhesion. Genetics, growing environment and harvesting all play a crucial role and we know that it is their interactions that are the most influential on skinning severity. The importance of the weather and how this interacts with different varietal susceptibility has also been reported (Brennan et al., 2017b; Froment & South, 2003).

1.3 Malting

1.3.1 Malting process

Malting is a process of controlled germination of grains, in which breakdown of the cell walls surrounding starch granules in the endosperm takes place. This is necessary in order for the starch to be available for conversion to maltose; this section introduces the steps involved in producing malt. The objective of malting is modification of the barley endosperm. 'Modification' is a term used to describe the changes in the endosperm observed during malting. These changes are: 1) production and release of hydrolytic enzymes, 2) breakdown of cell walls surrounding starch granules in the endosperm and 3) change of physical properties of barley grains, from hard and difficult to crush to brittle and floury. Well-modified grains have the cell walls broken down, but only a small proportion of starch (approximately 18%), which has been hydrolysed; the remaining quantity of starch will be broken down during

mashing in brewery or distillery (Briggs, 1998). Breakdown of starch, mainly into maltose, is necessary for the yeast to have a substrate to ferment into ethanol.

Malting has three distinct steps: steeping, germination and kilning. The malting process, in order to be considered efficient, needs to result in homogeneous malt, with all the grains modified efficiently and to the same extent. During steeping, the water is imbibed through the micropyle in the grain and it is then distributed through the endosperm (Rathjen et al., 2009). The grains are subjected to combinations of periods of submersion in water followed by air rests, over a period of up to 48 h. The objective of steeping is for the grains to increase in moisture content from approximately 12% to 42%. It is the increase in moisture and detection of the water by the embryo that starts the germination process. Levels of approximately 42% moisture content are optimal for even and uniform germination. The detection of water by the embryo resumes metabolic activity and hormonal signalling starts the germination. Gibberellic acid (GA) is produced in the embryo in response to the water imbibition and it diffuses through the aleurone layer promoting the production and release of enzymes and growth of root and acrospire (Clutterbuck & Briggs, 1973; Palmer, 1974). Simultaneously the levels of abscisic acid (ABA) are reduced. Abscisic acid is a hormone with opposite effects to GA; it is responsible for maintaining dormancy and preventing germination of the grains. Hormonal signalling is described in greater detail in section 1.3.2. Grains are steeped in aerated water, although it has been shown that aeration does not necessarily improve the homogeneity of germination (Kelly & Briggs, 1992a). The water temperature is held constant, usually at 16^{o}C, as this temperature has been shown

to result in slower germination than that in grain in high temperatures, but the germination process is even and production of hydrolytic enzymes is most efficient (Baxter & O'Farrell, 1980; Reeves et al., 1980; Agu et al., 2016).

Steeping is followed by grain germination, which usually takes four days, at a constant temperature of 18^{o}C. During this step grains are allowed to grow roots and acrospires, and enzymes are produced and released. These enzymes are necessary to break down the cell walls surrounding starch granules, and later during mashing for breakdown of the starch into simpler sugars, accessible to yeast to convert to ethanol. Mechanical agitation of grain during germination prevents the roots from clumping and 'hot-spots' from forming. 'Hot spots' are areas of raised temperatures within the grain bulk caused by biological activity, usually in regions inaccessible to standard temperature probes, and they result in increased risk of fungal infection and heat damage within the bulk of grains (Briggs, 1998).

The germination phase of malting is halted by kilning the grains. This usually takes place over 24 h at temperatures usually not higher than 70^{o}C, in order to preserve the activity of the enzymes produced during germination, but high enough to halt the modification of the endosperm, before its carbohydrate stores are used up in support of the plant growth. During kilning grains lose the moisture content and final kilned malt has approximately 4% moisture content. Grains after malting are very brittle and husks provide a 'package' for the endosperm, which at this point is easily broken.

In brewing and distilling dry malt is used as substrate for alcohol production. Malt is milled to a specified particle size, usually by a roller mill. This is then mixed with hot water, which has been set to optimal temperatures for enzymatic activity; this process is referred to as mashing. During mashing, enzymes produced in the malting process break down the starch into simple sugars, mainly maltose. In the brewing process when mashing is complete the liquid, called wort is separated from the insoluble part of the grain by filtering the grain through the grain bed. In contrast in distilling the mash (in distilling called the wash) is not filtered and the yeast is added directly to it. Good quality of malt in brewing will produce clear wort, ready for addition of yeast and fermentation, poor quality malt results in cloudy wort which is difficult to filter.

1.3.2 Enzymes in malting

Barley grains are metabolically inactive during the quiescent state and through the period of dormancy, a phase in seeds in which germination is prevented until the arrival of favourable environmental conditions, therefore maximising the chances of plant survival. In malting barley, dormancy is broken by the grains experiencing a warm dry period, called the after ripening period. Upon imbibition, grains resume their energy metabolism, protein synthesis and hormonal signalling. Abscisic acid and gibberellic acid are two main hormones implicated in the initiation of barley grain germination. Abscisic acid is responsible for maintaining the grains in the quiescent state and plays an important role in maintaining dormancy; during imbibition the

content of ABA in the grain reduces rapidly (Jacobsen et al., 2002), but also the embryo's sensitivity to ABA reduces (Barrero et al., 2009). Gibberellic acid production in the embryo during germination is triggered by reduction of sugars due to respiration (Perata et al., 1997), and GA diffuses along the aleurone layer triggering the release and production of hydrolytic enzymes including α- and β-amylase, β-glucanase, pentosanases, proteases, phosphatases and enzymes that degrade nucleic acids (Briggs, 1972; Groat & Briggs, 1969; Briggs, 1998). Accumulation of these hydrolytic enzymes, mainly α- and β- amylase, and breakdown of cell walls in the endosperm are the main objectives of malting, as they are required for starch breakdown during malt mashing.

Starch is the major component of barley grains and it constitutes up to 70% of the grain (Zhu, 2017). It is major storage carbohydrate, providing energy for germinating grains, before the emergence of green leaves. Starch in the mature endosperm is stored in the endosperm in the form of large A granules (approximately 25 µm in diameter), constituting 90% of the total granules and small B granules (approximately 5 µm in diameter) accounting for 10% of all granules (Bathgate & Palmer, 1973). Starch is a mixture of two polysaccharides amylose and amylopectin, and the proportion of each of these polysaccharides varies depending on the barley variety; a comparison of the two polysaccharides is presented in Figure 1.3. Amylose chains are mostly linear and consist of on average 2000 glucose residues linked at α-(1, 4) bonds. Amylopectin has much higher molecular weight than amylose, and in addition to linear α-(1, 4) bonds it has a lot of branches through α-(1, 6) bonds.

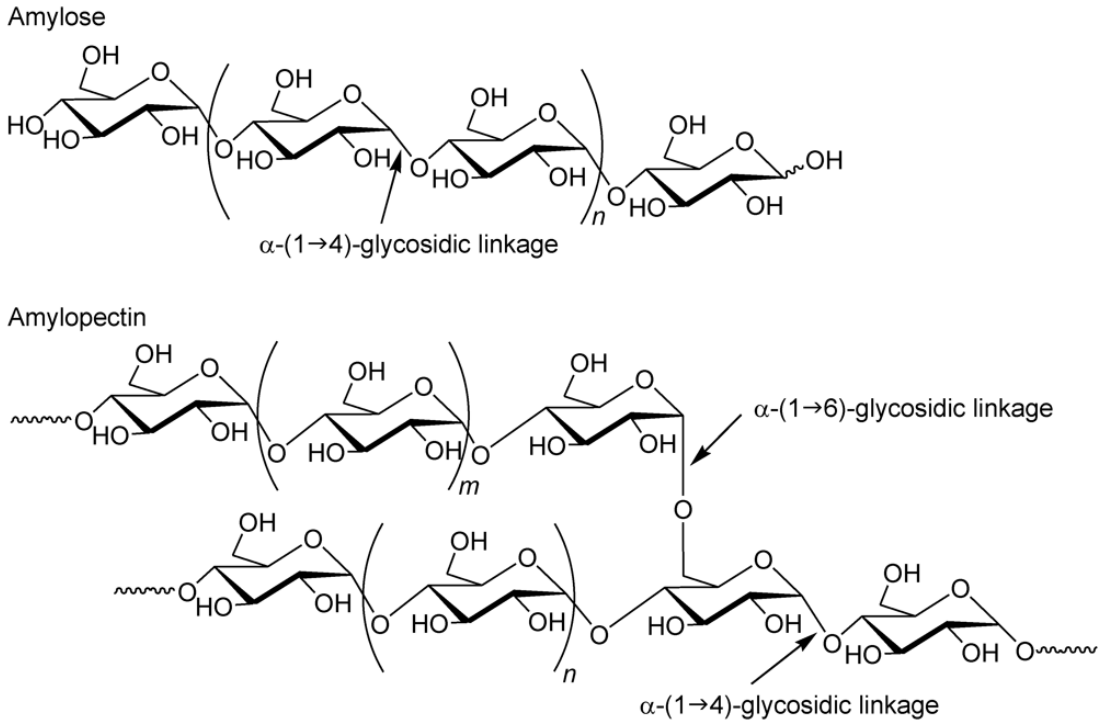


Figure 1.3 Molecules of amylose and amylopectin. Source: (Chemistry.stackexchange.com, 2016)

Starch granules in the endosperm are enclosed with cell walls, and a major component of the cell wall in the endosperm is (1,3;1,4)-β-D-glucan, which is hydrolysed mainly by (1,3;1,4)-β-D-glucan endohydrolases and β-glucanase (Burton et al., 2010). After hydrolysis of cell walls, enzymes responsible for starch breakdown begin hydrolysing the granules. Enzymes of most importance in starch breakdown are α- and β-amylase; α-glucosidase and limit dextrinase, schematic representation of the action of those enzymes on the starch molecules is presented in Figure 1.4.

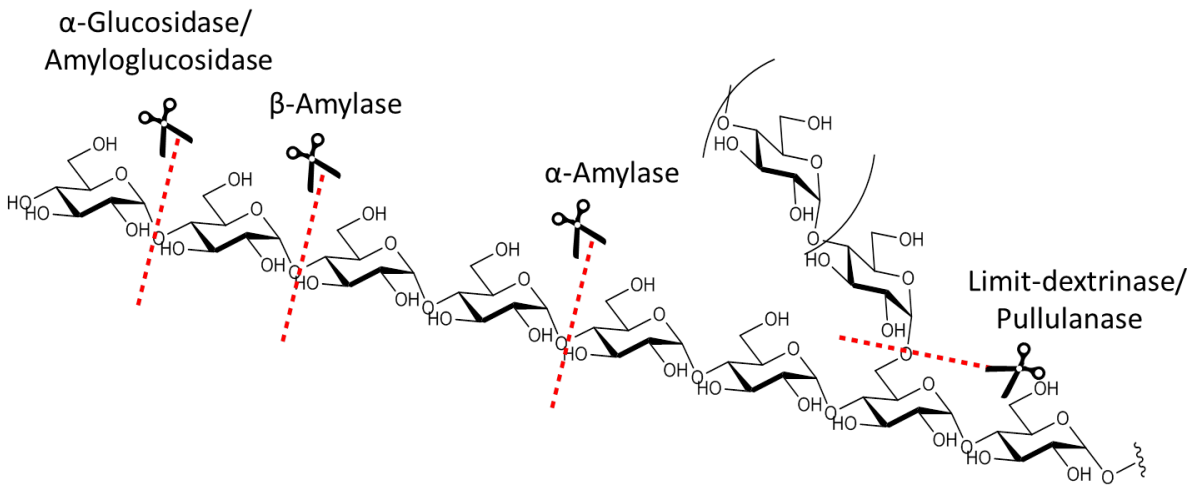


Figure 1.4. Schematic representation of the action of starch degrading enzymes on amylopectin molecule. Source: (Megazyme, 2019)

α-Glucosidase is an enzyme which breaks the disaccharide maltose into two glucose units; it can also remove the terminal glucose from the starch molecules. It is highly concentrated in the embryo and the aleurone layer. It accelerates the breakdown of starch granules and it is able to hydrolyse them on its own (Maeda et al., 1979).

α-Amylase is absent from dry barley grains, it is synthesised *de novo* after diffusion of GA through the aleurone layer. It is subsequently released into the starchy endosperm, it is an *endo*-acting enzyme and it catalyses the hydrolysis of starch at random points of the chains at α-(1, 4) glucose linkages. This enzyme on its own is capable of attacking and degrading starch granules producing a mixture of sugars, including glucose, maltose, oligosaccharides and dextrins.

β-Amylase, unlike α-amylase is present in abundance in the endosperm of mature barley grains, mainly in the sub-aleurone layer, however it is not active. It has

two forms, free and bound. Its bound form is linked with disulphate bonds to a specialised protein, called protein Z, and it is released by proteolytic enzymes produced during germination (Grime & Briggs, 1996). It is an *exo*-acting enzyme and on its own β-amylase is not able to degrade starch granules, it is however able to carry out a stepwise attack on amylose. It catalyses the hydrolysis of the α-(1, 4) linkages, second to the non-reducing chain ends, releasing maltose and an oligosaccharide shortened by two glucose residues.

The final enzyme of importance in starch breakdown is debranching enzyme, also called limit dextrinase, pullulanase or R-enzyme (Longstaff & Bryce, 1993). This enzyme catalyses cleavage of amylopectin at α-(1, 6) linkages, and by breaking branch points it facilitates the breakdown of starch by the other enzymes. Debranching enzyme occurs as an inactive zymogen, an inactive protein substance, requiring activation by another enzyme, in the endosperm of mature barley (Maeda et al., 1979). Large proportions of this zymogen may remain inactive at the end of malting (Longstaff & Bryce, 1993). The action of limit dextrinase during malting is also limited by heat-stable protein that inhibits malt limit dextrinase (MacGregor, 2004). MacGregor et al. (1999) found that efficiency of any of the starch degrading enzymes is influenced by the presence of other enzymes, and limit dextrinase significantly contributes to improvements in the efficiency of starch breakdown in mashing. However the lack of activation and the existence of the protein inhibiting the action of the limit dextrinase mean its contribution to improving malt quality is limited.

1.3.3 Effects of skinning on malting

Husk adherence and the husk content of the barley grain and its importance during malting were first researched from 1920. Harlan (1920) observed that husk adherence occurs 10 days post anthesis. Stevenson et al. (1930) is one of the earliest publications discussing the difference in husk content of different barley varieties followed by Malloch (1936) who noticed that poor storage and rough handling of the grains increases their susceptibility to damage. Reinbergs and Huntley (1957) first observed that different barley varieties have different strengths of husk adhesion and grains without husks during malting are susceptible to damage. They also described the difference between the growth of the acrospire in germinating barley in grains with attached husk and in skinned grains, increasing the susceptibility of skinned grains to damage. Malt produced from skinned grains was of poor quality and a large proportion of skinned grains in the sample resulted in microbial growth and unacceptable appearance due to excessive microbial infection (Meredith, 1959). More recent studies on husk adhesion concluded that the structure (shape and thickness) of the cells in direct contact with the adhesive layer play an important role in the quality of adhesion, and each step of malting process, including transport exacerbates skinning (Olkku et al., 2005). Modern barley varieties are more susceptible to skinning than older varieties, indicating the increasing importance of this problem and the need to further understand it (Brennan et al., 2017b).

Efficient malting produces good quality, homogeneous malt. Quality of barley grains, including the severity of skinning in the sample could impact malting process

at each of the three malting stages. During steeping, barley imbibes water and at this stage adherent husks are necessary to ensure even water uptake and distribution in the grains, as barleys which have lost their husk can take up water faster, modify faster and lead to overmodification and loss of valuable extract (Bryce et al., 2010). In addition, Dunwell (1981) and Lenoir et al. (1986) have shown that removal of husks in dormant grains alleviates dormancy and allows the grains to germinate, therefore implicating the husk in the germination inhibition. Alternatively if the malting regime is not adjusted to accommodate the faster germination of grains without husks, it could result in embryos being starved of oxygen and drowning. The hypothesis of barley grains drowning is supported by findings from experimental malting of naked barley grains. Fully skinned grains are similar in their appearance to the caryopsis of naked barley, also called hull-less, which does not have adherent outer husk. It does not produce the sticky cementing layer, and the lemma and palea are free threshing at harvest similarly to wheat grains. Agu et al. (2009) and Swanston & Middlefell-Williams (2012) were able to produce a good quality malt from skinned grains, but only after adjusting the steeping regime. The adjustment involves reduction in water, hull-less barley requires less water during steeping, the grains in excessive water are starved of oxygen and drown, similarly to what happens if large proportion of skinned grain in the sample is present. Both scenarios: faster germination and drowning would lead to production of inhomogeneous malt, which is undesirable by the maltsters and causes problems during processing, including issues during filtration and reduced recovery of extract from malt.

Skinning could have detrimental effects on modification during malting in the germination stage, as this process can only take place if the embryo is viable. Historical evidence suggests that intact husks are crucial in protecting the embryo from mechanical damage and grains without husks do not malt and modify fully (Reinbergs & Huntley, 1957; Meredith, 1959). The acrospire in intact grains grows under the lemma and is protected from damage during the agitation of malt. In contrast, in skinned grains it projects out from the grain; the difference in acrospire growth between skinned and unskinned grains is presented in Figure 1.5. In addition, the loss of husk could potentially cause a perceived increase in the extract recovered from malt, due to lower husk proportion in the bulk. This was first observed by Meredith, (1959), who corrected the malt extract calculation to account for the lost husk and found that extract recovered from skinned grains was lower than from intact grain. Swanston et al. (2011) also made an observation that absence of the husk in the sample inflates the extract values.

Figure 1.5. Acrospire (coleoptile) growth in a barley grain; a-unskinned barley, arrow - acrospires growing along the caryopsis under the husk; b-skinned grain, arrow - acrospire growing outwards from the caryopsis

After kilning, malted grains are very brittle and the husk provides packaging for the endosperm, which at this point is easily broken. In skinned grains this protection is compromised, and breakage of the endosperm during transport and handling results in production of dust, which is dangerous for the personnel handling it and needs to be extracted, adding costs to the production (Olkku et al., 2005).

Husks also play an important role in the process of wort filtration. After malted grains have been milled and mashed, husks form a filter bed, which aids in clarification of the wort. In distilling, peated whisky is produced by adding a proportion of malt which has been flavoured with peat smoke. Husks can play a role in binding the phenols from the peat smoke (during malt kilning). Phenols are one of the most important classes of compounds, giving peated Scotch whisky its unique flavour (Macfarlane, 1968; Macfarlane et al., 1973).

Studies agree that it is possible to produce malt of adequate quality from naked barley (Swanston & Middlefell-Williams, 2012; Swanston et al., 2011; Agu et al., 2008). However unlike hull-less barley, skinning affects only a proportion of the grains in the bulk, producing mixed bulks, and it is the combination of the different types that makes it less efficient to malt. Using standard programs designed for husked grains will result in incomplete modification of the skinned grains, and adjusting it will in turn not favour the grains with intact husks.

1.4 Thesis aims and structure

This thesis examines skinning from two perspectives: farmers and maltsters. The overall thesis aims are to investigate the influence of selected environmental factor (moisture) on the severity of skinning and quality of barley husk adhesion at harvest and to quantify the effects of skinning on the modification and homogeneity of the malt. Understanding of the impact of environmental conditions at harvest will contribute to developing the best practice for farmers to reduce the skinning in barley during harvesting of the grains, and for maltsters it quantifies the impact of skinning on homogeneity of malt produced. The understanding of the impacts of skinning could lead to development of malting processes suitable for bulks with large proportion of skinned grains.

Producing malt of the highest quality starts with harvesting the highest quality barley. The work presented in **chapter 2** examines the role of two types of pre-harvest grain moisture on husk adhesion. First type of moisture is associated with rainfall and second with stages of grain ripeness. The work also examines the type of tissue damage in skinned grains grown in the glasshouse and in the field, in order to determine the best harvest time and conditions to avoid or reduce skinning risk. **Chapter 3** quantifies the effects of the presence of skinned grains in malting batches of two commercially important varieties on malt quality. It investigates how this affects the standard methods of malt quality assessment used in the industry, including malt hot water extract and homogeneity. In depth investigation of the malt samples and levels of modification was conducted in **chapter 4**. This allows for better

understanding of the level of modification in the malted samples and for validating which of the two contrasting events, undermodification or overmodification of the grains is responsible for lack of homogeneity in malt produced from skinned grains. **Chapter 5** presents in detail how different forms of husk-loss e.g. partial and complete loss of husk influenced the modification. Water uptake, germination and enzyme production were measured in single grains with various husk-loss types.

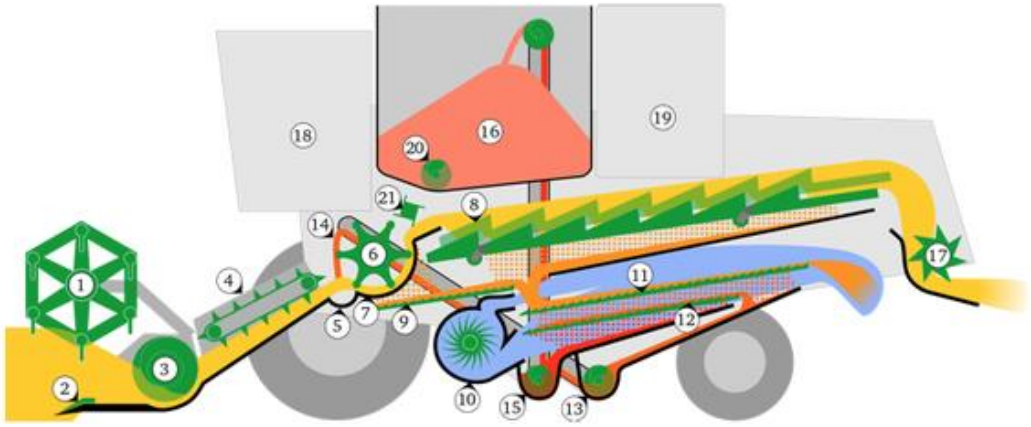
As a whole, the experimental work in this thesis was planned to give a better understanding of the causes of skinning and the influence of skinning on the quality of malt produced, as drawn together in final discussion in **chapter 6**. The approach to the experimental work from both farmers and maltster's perspective, could form a foundation for better communication on this issue, and could aid in formation of guidelines for both industries.

Chapter 2. Influence of moisture on the quality of barley grains in the weeks preceding the harvest

2.1 Introduction

The incidence and severity of grain skinning in malting barley is dependent on the combination of genetic and environmental factors. Molecular causes are described in detail in the literature review (Chapter 1); subsequently this chapter investigates one of the major environmental factors, which is hypothesised to influence skinning – grain moisture content, both during ripening and at harvest.

Harvesting is the time when skinning is most likely to become evident, as the combine harvester uses mechanical forces to separate grain from the rest of the plant. The combine harvester cuts the crop, which is then fed to the threshing drum, and passes through a series of conveyor belts and sieves. Crucial settings that are most likely to influence husk retention are the speed of the threshing drum and the distance of concave clearance around the rotating drum, and those are components numbered 6 and 7 in Figure 2.1.



1. Reel
2. Cutter bar
3. Header auger
4. Grain conveyor
5. Stone trap
6. Threshing drum
7. Concave
8. Straw walker
9. Grain pan
10. Fan
11. Top adjustable sieve
12. Bottom sieve
13. Tailings conveyor
14. Rethreshing of tailings
15. Grain auger
16. Grain tank
17. Straw chopper
18. Driver's cab
19. Engine
20. Unloading auger
21. Impeller

Figure 2.1. Diagram of combine harvester. Source: North Carolina Cooperative Extention (2019)

When the settings of the combine harvester are too harsh, the bulk could contain a large proportion of skinned grain. Equally if the settings are too gentle, the awns and straw will not be effectively removed; this increases the admixture in the bulk and adds extra handling costs before a sample can be accepted for malting. Although the harvesting process has a major impact on grain skinning, pre-disposition of ripe grains to skinning is likely to have been determined by a combination of genetic factors and growing environment.

A focus on the effects of grain moisture content on the severity of skinning is driven by anecdotal evidence that the risk of skinning may change, depending on the

environmental conditions prior and during harvest. Observations and reports made by farmers and maltsters indicate that skinning is more prominent in Scotland, which has colder, wetter and less sunny growing conditions than some regions in England or other European countries, and where this problem is less prevalent. It has been previously shown that grains exposed to a misting treatment in the glasshouse and in the field had significantly higher severity of skinning than control grains (Brennan et al., 2017b; Froment & South, 2003). In addition to misting treatments, Froment & South (2003) have found in their field trials that sink limited grains, in which top half of the ears was removed, significantly increased in skinning severity in the malting barley variety Chariot. These authors suggested that larger grains from the sink limited treatment contributed to a 'mismatch' between the sizes of the caryopsis and of the husk, thus weakening the strength of adhesion; this hypothesis was also supported by Rajasekaran et al., (2004). However this was not confirmed by Brennan et al. (2017a) who found no relationship between caryopsis size, husk dimensions, and the severity of skinning.

The increase of grain skinning in regions such as Scotland could be a consequence of a cooler and wetter, or intermittent wet-dry climate. It is therefore expected that changes in grain moisture, either during the ripening period or at harvest time would explain variation in skinning severities. There are two different aspects of moisture content affecting grain quality: (i) the rate at which grain moisture changes during the ripening period and (ii) the influence of environmental moisture from rain and humidity on the grain during ripening or when harvest ripe.

Changes in grain and/or environmental moisture could also affect adhesion and retention of the husk.

It is possible that two separate mechanisms of barley grain skinning are responsible for husk-loss of the grains. It was first speculated by Gaines et al. (1985) that the separation of the husk and caryopsis in skinning occurred along the cementing layer. Later study by Olkku et al. (2005) showed evidence of breakage of parenchyma cells of the husk in the skinned grains, however the details of the growing environment of the grains used for light microscopy in this study were not described. Misting treatment in Brennan et al. (2017) also showed significant increase in grain skinning without affecting other barley ear characteristics including ear length, floret number, grain number or grain weight. It was suggested that changes of the cuticular cementing layer are responsible for the increase in skinning. Brennan et al. (2017a) have also shown that warm pre-anthesis and cool post-anthesis temperatures increase the severity of skinning by altering the composition of the cuticular cementing layer. Cementing layer is produced by the pericarp and it is cuticular in origin, similar to the cuticles of other fruits. Previous work on fruit suggests that environment affects plant cuticles, exposure of the surface of the sweet cherry fruit to water causes microcracking (Knoche & Peschel, 2006). Similarly excess water and low night temperature during development of peppers cause microcracking of the cuticular membrane, which eventually causes cracking of the pericarp (Aloni et al., 1998).

It is possible that the cementing layer behaves in a way similar to other adhesives commonly used in material sciences, in which moisture and humidity weaken this layer by permeating the adhesive and displacing it at the bonding site (Ebnesajjad & Landrock, 2009). However there are two different aspects of moisture content affecting the grain quality: firstly, the environmental moisture from rain and humidity and secondly, moisture content associated with the growth stage of the plant and the ripeness of the grain, as the grains which are green and soft also have high moisture content.

Development of all cereals, including barley can be classified into growth stages (GS) using a decimal code. This code has been first developed by Zadoks et al., in 1974, and prior to this both research findings and timing of treatments have been described imprecisely. Following this initial classification a more robust and precise decimal description has been developed by Tottman & Broad (1987), based on the Zadoks scale. This decimal code is universally used in cereal experiments and treatment recommendations, including applications of herbicides, pesticides or fertilisers. In this chapter most of the experiments were conducted toward the end of the development of the barley plant between GS 87 (hard dough) and GS 92 (caryopsis hard) (Tottman & Broad, 1987), and exact growth stages are referred to in the description of the individual experiments.

Firstly investigation into the effect of very small quantities of environmental moisture at the last stage of barley growth was carried out. This was investigated in barley around GS 92, just before harvest or possibly in a situation where rain starts

to fall during harvest. In this experiment a period of wetting, followed by air rest periods were used. These wetting periods translate to rainfall in the field with air rest being a period of time after the rain. Although the exposure to water was only one minute, the whole grain was submerged under water, therefore requiring a shorter exposure than it would to a rainfall. The choice of varieties for the investigation was guided by Brennan et al. (2016), who has investigated 200 spring barley varieties and their skinning susceptibilities. The varieties chosen in this chapter had varying susceptibilities to skinning ranging from resistant to very susceptible. In addition use of grains that were ripe and pre-harvested, simulated the situation of rainfall on the barley in the field on the day of harvest. The understanding of this would help farmers make a decision when to harvest and whether to carry on during a rainfall or stop the harvest.

Secondly, it was important to look at a longer period of rain during the harvest of fully ripe grains around GS 92, which would be a situation more commonly observed in Scotland. Misting experiments simulated rainfall, where ripe grains on the plant were exposed to water followed by air rest for up to five hours. Farmers might often be working against the clock and need to harvest the grains as soon as possible.

In addition to the above experiments farmers might often be tempted to harvest the grains early, before the bad weather sets in. This scenario was investigated in the glasshouse and in the field experiments, where barley grain at various stages of ripeness were harvested to establish whether harvesting too early,

grains that are not fully ripe can be detrimental to skinning. This experiment was also replicated in the field, where all the environmental factors of that particular season were at play and similarly to the glasshouse experiment the range of natural variation in ear ripeness was captured.

Through an understanding of the environmental impacts on skinning, especially the influence of moisture, it is hoped that guidance could be given to farmers. This would support their decision making on the optimal harvest time to achieve the highest quality grains and ensure their suitability for malting and therefore premium prices.

2.1.1 Aim and objectives

The aim of this chapter was to investigate the influence of moisture associated with weather conditions and with grain ripeness on grain skinning severity, in order to determine the best harvest time and conditions to avoid grain skinning. The objectives were: i) to examine how short and long soaking influences skinning in dry and harvest ready grains by soaking or misting the grains; ii) to investigate how moisture content associated with earlier growth stage and unripe barley grains impacts on severity of skinning in two different growing environments: glasshouse and field; iii) to examine the skinning mechanism and affected tissues in skinned barley grains using light microscopy.

2.2 Materials and methods

2.2.1 Plant growth

Glasshouse conditions were the same for all of the plant material grown. Seeds were planted in 4-liter pots, in which seven grains has been planted. The average temperature in the glasshouse compartment throughout the growing period was 18^{o}C, with minimum temperatures not dipping below 10^{o}C, and the light was supplemented by mercury vapour lamps to give a minimum of 16 h daylight period. The plants were watered into the pot directly, taking care not to wet the grains. Plants for each of the experiments were grown in a block design; each block was repeated three times.

2.2.2 Barley soaking experiment

Experimental design

The protocol for the experiments was developed using variety Propino grown in the field. Exactly 5 g of grains was submerged in water and the increase in the moisture content (MC) through increase in weight of the sample was measured every five minutes. This measurement was repeated three times. There was no increase observed for the first 10 min, first increase was observed at 15 min. Therefore it was assumed in the experiments described below that a long soak (10 min) did not affect

the MC. This section describes two separate experiments, both of which used the same experimental setup described below, and different treatment combinations.

Plant Material for experiments 1 and 2

Four barley varieties were selected for this experiment, depending on their skinning susceptibilities, as identified by previous project (Brennan et al., 2016). Varieties used were Henni (resistant to skinning), Astoria (mildly susceptible), Braemar (susceptible) and Propino (very susceptible); the average skinning scores and moisture contents for untreated grains (control) are in Table 2.1. Fresh plant material was grown for each of the two experiments. Barley ears were hand harvested at maturity and hand threshed, which involves carefully cutting off the awns and removing each grain from the rachis, detailed barley anatomy is discussed in Chapter 1.

Table 2.1. Mean skinning and moisture contents ± standard deviation were measured for untreated control grains for experiment 1 and 2. Expected skinning is based on research by Brennan et al., (2016)

Variety	Skinning (%) ± SD	Moisture (% WB) ± SD	Expected Skinning (%)
Henni	0.2 ± 0.5	11.74 ± 0.05	1.8
Astoria	16.7 ± 6.6	11.56 ± 0.04	1.6
Braemar	28.5 ± 3.2	11.62 ± 0.05	49.0
Propino	40.9 ± 6.1	11.12 ± 0.03	38.1

Treatments

Treatments in both of the experiments were a combination of wetting (or soaking) time followed by a period of air rest. In each block, grain from one ear per pot was

subjected to each of the treatments. There were in total five ears per block and the experiment was run in triplicate, giving a total of 15 ears subjected to each treatment. Experiment 1 consisted of different lengths of soaking followed by an air rest time, as described in Table 2.2

Table 2.2. Treatments in experiment 1, combination of wet and air rest time.

Treatment	Wet time (min)	Air rest time (min)
Control	0	0
W1AR1	1	0
W1AR30	1	30
W10AR1	10	0
W10AR30	10	30

Experiment 2 consisted of very short wetting period followed by air rest up to six hours, the times of soaking and air rests used in this experiment is presented in Table 2.3

Table 2.3. Treatments in experiment 2, longer air rest, following a short soaking.

Treatment	Wet time (min)	Air rest time (min)
AR1	1	1
AR30	1	30
AR60	1	60
AR120	1	120
AR240	1	240
AR360	1	360

The grain preparation and handling procedure was the same for both experiments. Small aluminium boats were filled with tap water at 18^{o}C, fresh water was used for each treatment. Experiments were conducted one replicate at the time, completing the series of treatments on all varieties and pots in one replicate before beginning the next one.

Following the treatments the grains were surface dried with filter paper and threshed one ear at a time in the Wintersteiger LD 180 (Wintersteiger, Reid, Austria). The thresher was set to 1 (lowest speed possible) for 10 seconds. Grains were scored for skinning using the standard assessment procedure described in section 2.2.7.

2.2.3 Barley misting experiment

Plant material

Two varieties on opposing ends of the skinning susceptibility scale were used: Propino (susceptible) and Henni (resistant) were grown in the glasshouse until full maturity, using the methods described in section 2.2.1. Each block consisted of four pots from each variety and two ears from each pot were selected after each treatment; each block was replicated in triplicate.

Treatment

On the day of harvest the plants were subjected to a misting treatment, similar to the one described by Brennan et al., (2017). Treatments consisted of 30 min misting

followed by 30 min of air rest, for a total of five hours. After each air rest two ears were harvested; control ears were fully ripe grains, harvested before the treatment commenced.

Harvest and processing

Each harvested ear was split along the rachis; half of the grains was used for determination of grain skinning by threshing in the Wintersteiger LD 180 thresher (Wintersteiger, Reid, Austria), set to lowest speed possible (setting 1) for 10 seconds. After threshing grains were scored for skinning using the protocol described in section 2.2.7 . The other half of the grains was use for determination of moisture content using the method described in section 0.

2.2.4 Glasshouse moisture content experiment

Plant material

Four barley varieties with varied susceptibilities to skinning were selected for this experiment, as identified by previous project (Brennan et al., 2016). Varieties used were Henni (resistant to skinning), Astoria (mildly susceptible), Braemar (susceptible) and Propino (very susceptible). Plants were grown as described in section 2.2.1 and ears were harvested at different stages of ripeness, resulting in varying moisture contents associated with earlier/later growth stages. Each block consisted of five pots

of each variety and this experiment was run in triplicate; each replicate was planted approximately 7 weeks apart.

Harvest and processing

Barley ears were harvested close to full maturity, at growth stage (GS) 89-92 (Tottman & Broad, 1987). Grain ripeness varies naturally within the ear, allowing for wider spectrum of MC to be captured. Harvested barley was processed on the same day. Each ear was split longitudinally; half of the grains was used for determination of grain skinning by threshing in the Wintersteiger LD 180 thresher (Wintersteiger, Reid, Austria), set to lowest speed possible (setting 1) for 10 seconds. After threshing grains were placed back in labelled plastic bags and scored for skinning using the protocol described in section 2.2.7 . The other half of the grains were used for determination of moisture content using the method described in section 2.2.6.

2.2.5 Field moisture content experiment

Plant material and growth

Three sites were chosen in different parts of Scotland, maximising the variance in the weather and soil conditions, thereby maximising the range of potential grain moisture contents. The sites were Gilchriston in East Lothian (55°51'25.4"N 2°52'24.8"W), Boghall Farm in Edinburgh (55°52'26.0"N 3°12'26.7"W) and Drumalbin in Lanark (55°37'13.9"N 3°44'30.1"W) in 2017.

Table 2.4 Average annual temperature and precipitation over 30 years from each of the field trial sites. Source MET Office (2020).

Site	Weather station	Annual precipitation (mm)	Annual temperature Min (°C)	Annual temperature Max (°C)
Gilchriston	Dunbar	603.5	6.2	12.5
Boghall	Penicuik	980.3	4.2	11.8
Drumalbin	Drumalbin	900.3	4.5	11.2

Three varieties of spring barley with varying degree of skinning susceptibility were grown, in brackets levels of expected skinning are stated as described by Brennan et al.,(2016): Propino (highly susceptible (38.1%)), Concerto (medium susceptibility (16.4%)) and Westminster (low susceptibility (9.2.%)). The plot size was 10 m x 2 m; dates of sowing sampling and harvesting are presented in Table 2.5. The seed rate was 360 seeds/m^2 at Drumalbin and Boghall, and 340 seeds/m^2 at Gilchriston.

Boghall plots have received a fertiliser shortly after sowing. The applications were 60 kg/ha of nitrogen, 60 kg/ha phosphorus and 60 kg/ha potassium and second application of 60 kg/ha of nitrogen and 10 kg/ha of sulphur. Herbicides applied on these plots were high load Mircam at 1.0 litre/ha; Ally 30 g/ha and Compitox plus 0.25 litre/ha and fungicide program of: 0.5 litre/ha Siltra, 1.0 litre/ha Bravo and 0.3 litre/ha at GS 51-59.

Gilchriston plots received the first fertiliser application shortly after sowing. The applications were of 60 kg/ha of nitrogen, 60 kg/ha phosphorus and 60 kg/ha potassium and second application of 70 kg/ha of nitrogen. Herbicides applied on

these plots were High Load Mircam 1.0 litre/ha and Compitox plus 0.25 litre/ha and full fungicide treatment of: Flexity 0.25 litre/ha and Comet 0.4 litre/ha at GS 25-30 and Vivid + 0.4 Bravo (1.0) at GS 49. Manganese at a rate of 1 litre/ha was also applied at GS 12 and 23.

At Drumalbin site the fertiliser applications were a split of 60 kg/ha of nitrogen in the seedbed followed by 35 kg/ha of nitrogen at GS13 and the herbicide used was Compitox Plus at 0.6 litre/ha, High Load Mircam at 1.25 litre/ha and Concert at 60 g/ha at GS 22-25. Fungicide treatment applied was Proline at 0.36 litre/ha and Comet 200 at 0.4 litre/ha at GS 25-30 followed by application of Bravo at 1.0 litre/ha and Comet 200 at 0.4 litre/ha at GS 45.

Table 2.5. Dates of sowing, sampling and harvest for each trial plot

Field	Sowing	Sampling	Harvest
Gilchriston	28/03/17	15 & 21/08/17	28/08/17
Boghall	29/03/17	24/08/17	01/09/17
Drumalbin	03/04/17	06/09/17	23/09/17

Plant harvest and processing

The sampling time of the sites aimed at capturing natural variability in the maturity of the grains. On the mornings of sampling, 40 ears from each variety was collected at random from across the whole plot at each site. Samples were brought back to the lab, where they were processed on the same day, as described below. Each ear was split along the rachis; half of the grains was used for determination of grain skinning

by threshing in the Wintersteiger LD 180 thresher (Wintersteiger, Reid, Austria), set to the lowest speed possible (setting 1) for 10 seconds. After threshing, grains were placed back in labelled plastic bags and scored for skinning using the protocol described in section 2.2.7. The other half of the grains was use for determination of moisture content using the method described in section 2.2.6.

Sampling from Gilchriston in East Lothian was done on two occasions as the first collection happened after a heavy overnight rainfall, which in normal circumstances would prevent harvesting of this field on that day.

2.2.6 Moisture content

To measure moisture content, the ASAE S35.2 method was modified to reduce the required sample size of unground grain from 10 g to 1 g. A bulk of Concerto grains was used to determine whether the modified method gave the same results as the original. The difference in moisture contents between large and small sample sizes was not statistically significant (P >0.05), as determined by an independent t-test. In the final method, 1 g of unground grain was placed in aluminium weighing dishes and dried at 130^{o}C for 20 h. The dishes were cooled in a desiccator over indicating silica gel, and then weighed (Sartorius GMBH, Göttingen, Germany, accuracy: 0.0001g). The percentage of moisture on the wet basis (wb) was calculated by dividing the loss in weight by the weight of the original sample and multiplying by 100.

2.2.7 Skinning assessment

All samples were screened over a 2.5 mm sieve to remove small grains prior to assessment. Grain skinning was scored using a protocol developed by SRUC and the Institute of Brewing and Distilling (Scottish Micromalting Group, The Maltsters' Association of Great Britain, Nottinghamshire, UK). Each grain was examined on both the ventral and dorsal side and grains with overall husk-loss exceeding 1/5 of the total surface area were scored as skinned. Skinning assessments and experimental work were carried out by the same researcher, to reduce the variance observed between assessors.

2.2.8 Skinning mechanism

In preparation for light microscopy grains of the variety Propino from the glasshouse experiment (26 grains) 'Glasshouse MC' described in section 2.2.4 and from each of the sites from field experiment 'Field MC' described in section 2.2.5 (39 grains) were cut into small segments using a razor blade and forceps. Cut sections were fixed in a freshly made solution of 2% paraformaldehyde and 0.1% glutaraldehyde in 100 mM sodium PIPES buffer (pH 7.2). The tissue was fixed for a minimum of four hours at room temperature to allow the fixative to properly infiltrate the tissue, after which it was stored in the fridge at 4°C.

To embed the tissue in resin it was rinsed in 100 mM sodium PIPES buffer (pH 7.2) five times to remove the fixative solution followed by dehydrate in an ethanol

series (30, 50, 70, 90, 95 and twice at 100%) for 15 minutes each time. Ethanol was replaced with LR White (London Resin Co. Ltd., Basingstoke, UK) by incubating the tissue with 100% ethanol and LR White (2:1 v/v) for one hour at room temperature, 100% ethanol and LR White (1:2 v/v) for one hour at room temperature, and then in pure LR White overnight (18 hours) at room temperature. Fixed tissues were transferred to cavities in PTFE mould, partly filled with resin and filled to the top after transferring the tissue. A Thermanox coverslip was used to cover the sections, paying close attention to removing all air bubbles beneath the coverslip to ensure proper polymerisation of the resin. Samples were polymerised in the oven overnight (24 hours) at 55°C. After removing from the oven, flat pellets of embedded tissue were placed in a gelatine capsule and filled with LR White using a Pasteur pipette and placed in a 96-well plate to keep them upright, and polymerised overnight (24 hours) at 55°C. This ensured that the plant material was in the correct orientation for sectioning as described below. When the resin had polymerised, the gelatine capsules were removed from the resin-embedded material using a razor blade and cutting board.

Embedded grains were sectioned to 1 µm thickness on Leica Ultracut ultramicrotome (Leica Camera AG, Wetzlar, Germany) and stained with 1% Toluidine Blue O in 1% aqueous sodium borate (borax), and viewed under a light microscope.

Iit is unclear which of the layer in barley grains is weakened in grains with high skinning susceptibility. In this experiment barley grains from the same variety

(Propino), grown in different environments (glasshouse and field) were examined under light microscope, allowing for examination of the tissues damaged.

2.2.9 Statistical analysis

Statistical analyses were conducted using R programming language (R Core Team, 2016) and package *lme4* (Bates et al., 2015) to fit linear models. A linear mixed effects model (LME) was used to assess the effects of treatment and variety (predictor variables) and their interaction on skinning levels (response variable) in barley soaking experiments. The blocking parameters were used as the random effects, where pot was nested within the row. The minimal adequate model to use was obtained by using a series of ANOVA comparisons.

A generalised linear model (GLM) was used to assess the effects of moisture content of the barley grains on the skinning severity in the barley misting, glasshouse moisture content and field moisture content experiments. The effects of moisture content (predictor variable) on severity of skinning were investigated. The logit link function was used to relate the proportion of skinned grains (response variable) to the predictor variables.

The significance of the difference between the growing environment (predictor variable) and the skinning mechanism in barley grains (response variable) in the 'investigating the skinning mechanism experiment' was tested with chi square statistical test.

2.3 Results

2.3.1 Barley soaking experiment

Experiment 1

A linear mixed effect model was used to determine the effects of the treatment and variety on levels of skinning in barley. The interaction of the treatment and variety was highly significant (P <0.001), although each of the varieties except for Henni follows the same pattern: short soaking up to 10 minutes improves the skinning scores and air rest further progresses this improvement. This pattern was not observed for Henni, because its control skinning score was very close to zero and even though the mean values for the skinning in longer soaking have increased, they were not significantly different to the control the results are presented in Figure 2.2 Model results of the impact of short and longer wetting (W) and air rest (AR) treatments on skinning in four barley varieties with various skinning susceptibility; the time (min) of wetting/air is described in the legend. The estimated means and 95% confidence intervals are plotted. Treatments sharing a letter are not significantly different from each other (P <0.05).. The significance levels between treatments and varieties are not described, as only differences within variety were of interest in this experiment. All the treatment in varieties Astoria and Braemar produced results significantly lower than the control (P < 0.05) and none of treatments was significantly different from each other, even though the mean values for longer

soaking were higher than for the treatments with short soaking. Propino was the only variety which had significantly higher (P <0.05) skinning scores for the treatment with longer soaking and long air rest, but this was still significantly lower that the control (P < 0.05). Longer soaking reduced skinning in Astoria, Braemar and Propino and caused the resistant variety Henni, which did not skin in control and short soaking to skin. An air rest after the longer soaking exacerbated the skinning severities in all the varieties; this however was not significant (P > 0.05). An observation was also made that the type of skinning differed between control and longer soaking categories: in control the husk breaks in small pieces and grains soaked for longer periods lose the whole lemma in one large piece.

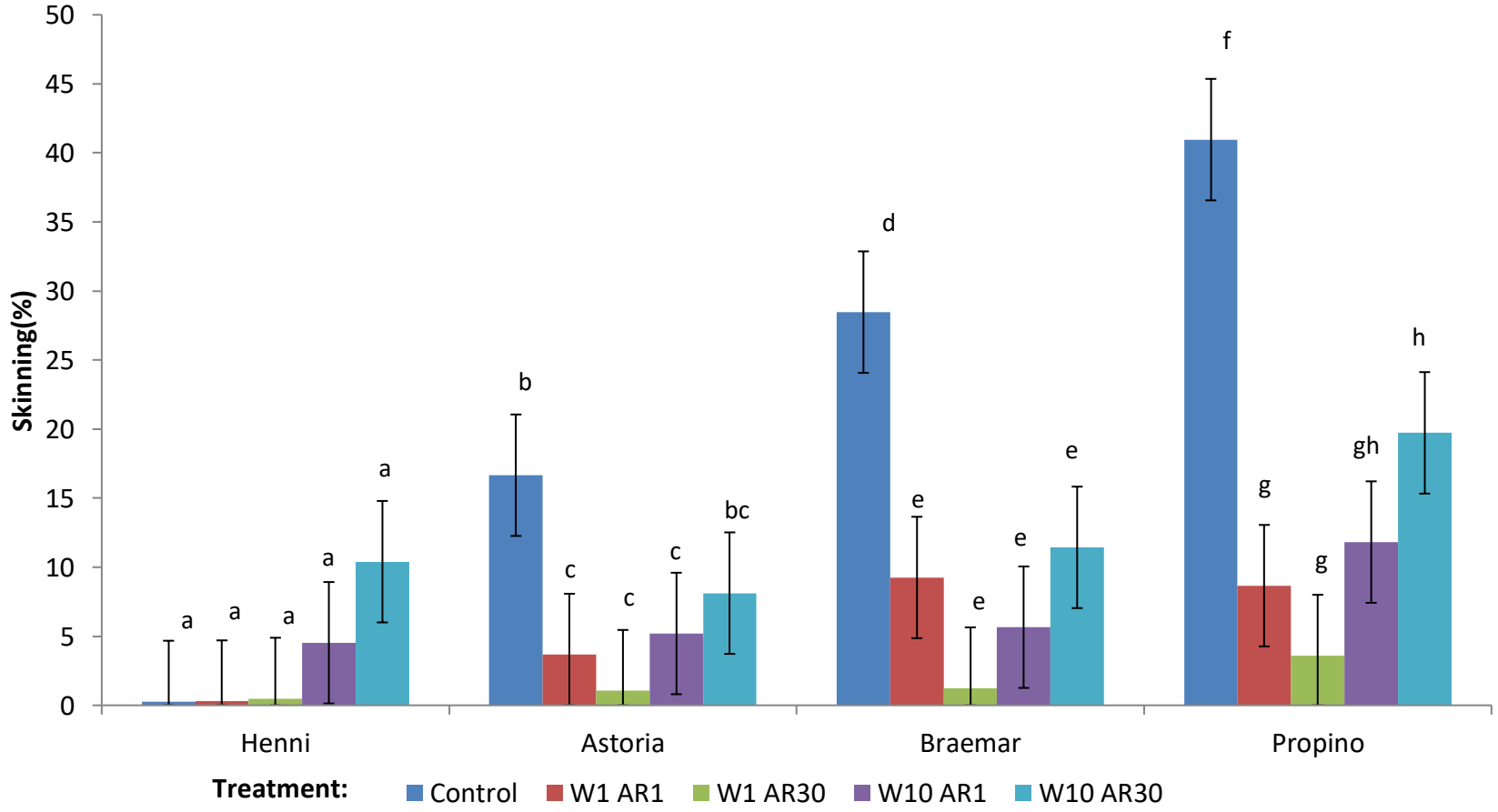


Figure 2.2 Model results of the impact of short and longer wetting (W) and air rest (AR) treatments on skinning in four barley varieties with various skinning susceptibility; the time (min) of wetting/air is described in the legend. The estimated means and 95% confidence intervals are plotted. Treatments sharing a letter are not significantly different from each other (P <0.05).

Experiment 2

In experiment 2 longer air rest periods following a very short exposure to water were investigated and analysed using linear mixed effect models and detailed results are presented in the Figure 2.3 The interaction of the variety and treatment was highly significant (P <0.001). Unlike experiment 1 in this case control grains of Braemar had the highest skinning scores, but they were not significantly different to untreated control grains of Propino (P >0.05). Untreated control of Henni and Astoria were significantly different to each other and to Braemar and Propino (P <0.05). Similar to experiment 1, Henni did not skin following short exposure to water, and no improvements were observed as the skinning score was already very close to zero. The three remaining varieties did improve with short soaking and as seen in experiment 1, this improvement was greater after the 30 min air rest period. Astoria showed a further slight improvement in skinning after a one hour air rest, but this was not statistically significant (P >0.05). Braemar and Propino show no further improvements and the skinning scores worsening from that time point for Astoria, Braemar and Propino and after six hours air rest all this three varieties have skinning scores not significantly different to the control scores. Astoria and Propino reach this level after four hours of air rest but Braemar was still significantly lower (P < 0.05) after four hours air rest than the untreated control, reaching levels similar to untreated control only after six hours air rest.

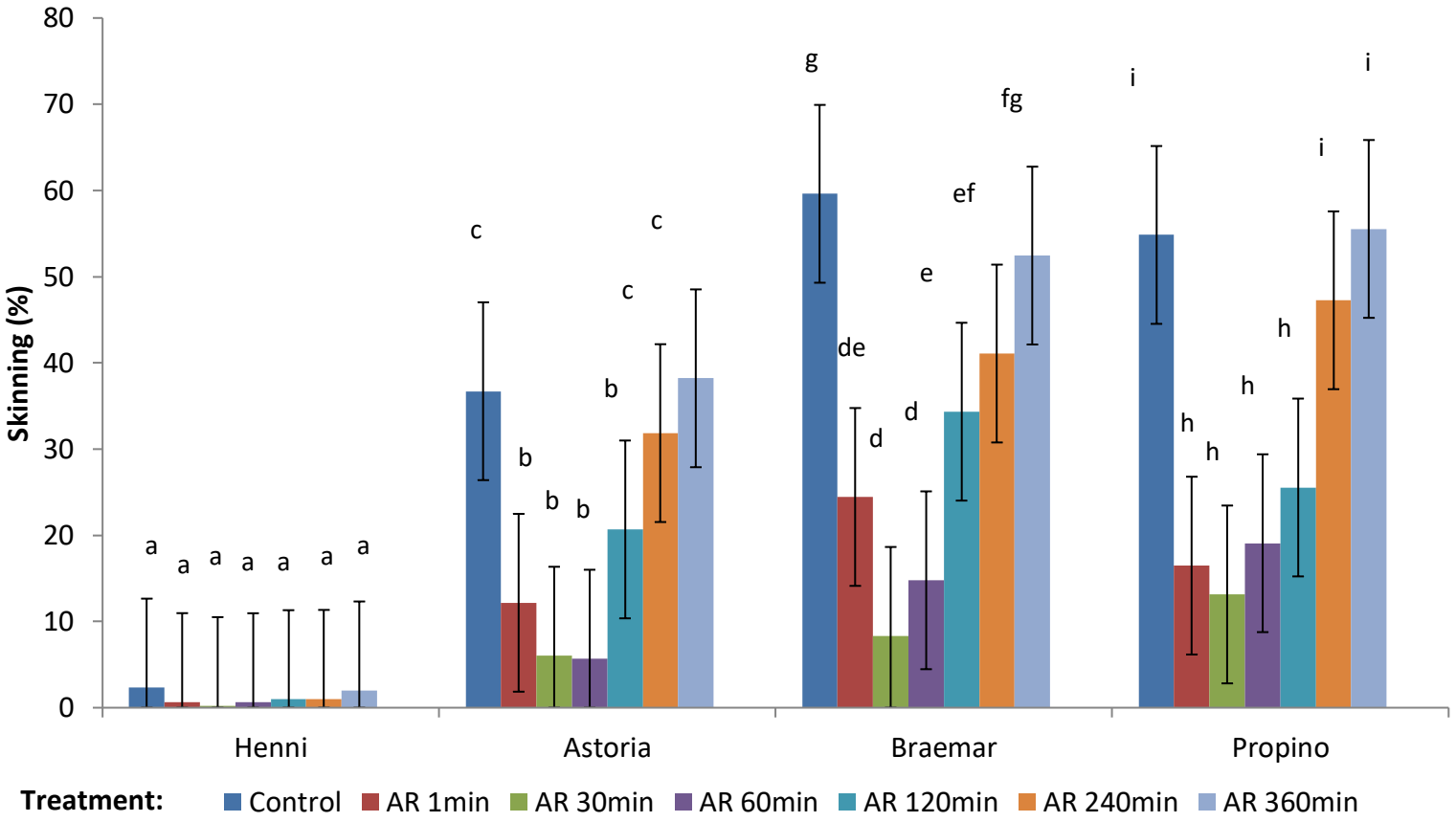


Figure 2.3. Model results of the experiment 2 on the impact of very short wetting and air rest (AR) treatments on skinning in four barley varieties with various skinning susceptibility. The estimated means and 95% confidence intervals are plotted. Treatments sharing a letter are not significantly different from each other (P <0.05).

2.3.2 Barley misting experiment

The number of 30 minute periods that the plants were misted for in each treatment (T0 to T5) were designed to produce a range of moisture contents in barley grains and details of the treatments and average moisture contents for each variety are presented in Table 2.6.

Table 2.6 Misting treatments of two barley varieties (Henni and Propino) at five time points

Variety	Treatment	Total Misting time* (min)	Moisture (%) (wb) ± SD
Henni	T0	0	12.64 ± 0.58
	T1	30	13.91 ± 1.71
	T2	60	15.20 ± 2.74
	T3	90	14.71 ± 1.78
	T4	120	17.73 ± 3.66
	T5	150	17.70 ± 3.43
Propino	T0	0	13.02 ± 0.64
	T1	30	14.28 ± 0.87
	T2	60	14.97 ± 1.54
	T3	90	16.89 ± 3.57
	T4	120	17.73 ± 3.74
	T5	150	19.58 ± 4.73

**total time includes only the misting time and does not include the air rest time*

The effect of long exposure to water on the skinning severity show large difference in the response of the two different varieties and are presented in Figure 2.4. Henni is an exceptionally resistant variety and in normal conditions it does not skin. However, with the increase in the grain moisture content an increase in skinning is first observed at approximately 17% moisture content (wb), in this case the

simulated rainfall has caused a resistant variety to skin. Propino is a variety that has extremely high skinning susceptibility and the results suggest that moisture has only a very slight impact on that, and grains with high moisture contents increased in skinning severity scores from approximately 50% for T0 plants to 55% for the grain with high moisture of over 25% (wb).

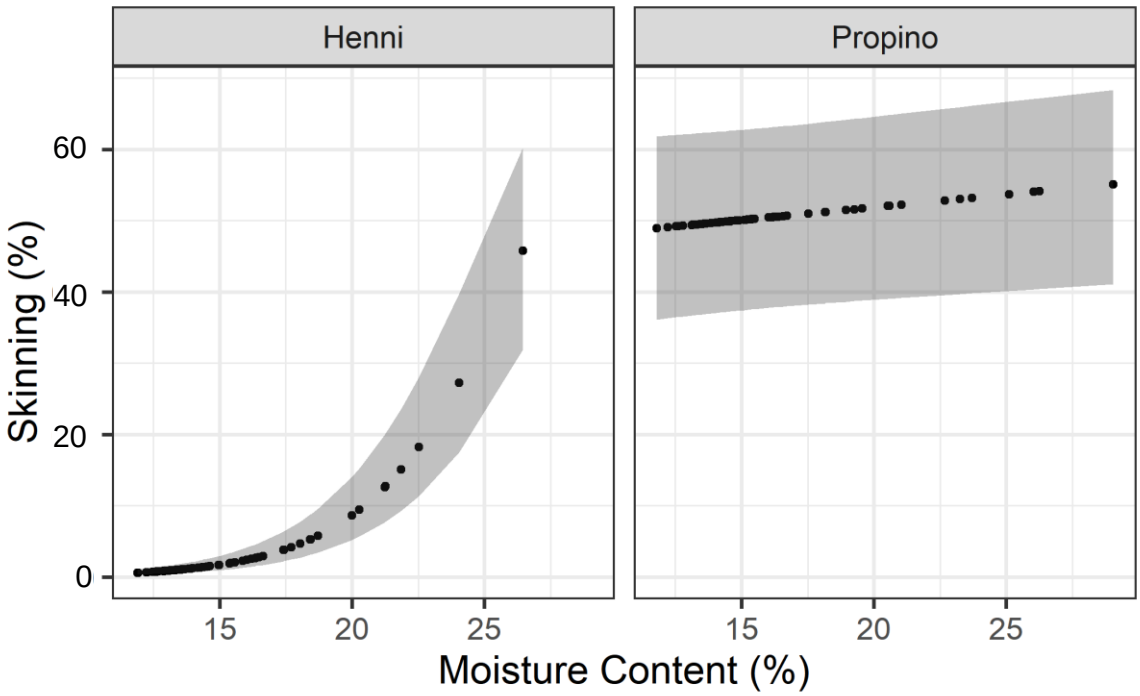


Figure 2.4. Barley misting results of the influence of moisture content on skinning severity; grey ribbon represents 95% confidence intervals.

2.3.3 Glasshouse moisture content experiment

A generalised linear model (GLM) was used to investigate the relationship between skinning and grain moisture content among varieties. An increase in moisture content significantly decreases the severity of skinning (P <0.0001); the relationship between MC and skinning is shown in Figure 2.5. All of the varieties had the same skinning response to the increase in moisture content associated with grain ripeness and all four varieties had lower skinning severities in barley ears with less ripe grains, close to 0% for all four of them, at moisture contents of approximately 35% (wb). Henni had skinning levels of 0% at lower moisture content than other varieties (approx. at 18% (wb)); however the level of skinning in the control grain was much lower than other varieties. Grains of the exceptionally good variety Henni had an even further reduction in skinning susceptibility from already low approx. 8% to close to 0% associated with growth stage moisture content.

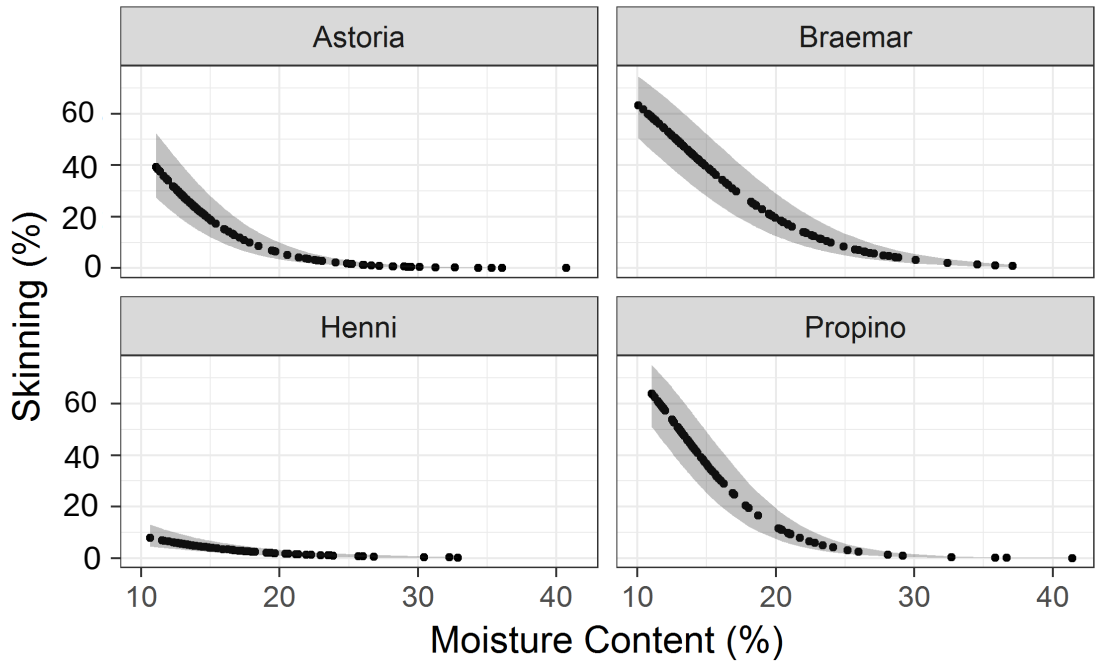


Figure 2.5. Influence of the moisture content associated with grain ripeness is barley grains grown in the glasshouse; grey ribbon represents 95% confidence intervals.

2.3.4 Field moisture content experiment

A generalised linear mixed effects model was used to determine the impact of moisture content on skinning severity in barley grown in the field; the results are presented in Figure 2.6. Three varieties had different susceptibilities to skinning. Westminister had the lowest, Propino the highest and Concerto usually placed in the middle. All three varieties had an increased skinning severity with increased moisture content of the grains; however the varieties differed in the level of moisture required for skinning severity to increase. Westminister had increased skinning at MC levels of approximately 40% (wb) whereas Propino had the sharpest response to the MC and ultimately the highest levels of skinning observed, with response of Concerto again

placing in between the other two varieties. The increase in skinning severity for Concerto was not as sharp as for Propino but also not as slow as for Westminster. The highest skinning severity was observed for Propino at around 80% skinned at moisture contents over 65% (wb). Highest moisture content for Concerto and Westminister was approximately 55% (wb) with highest skinning of 37% and 23% for those varieties, respectively.

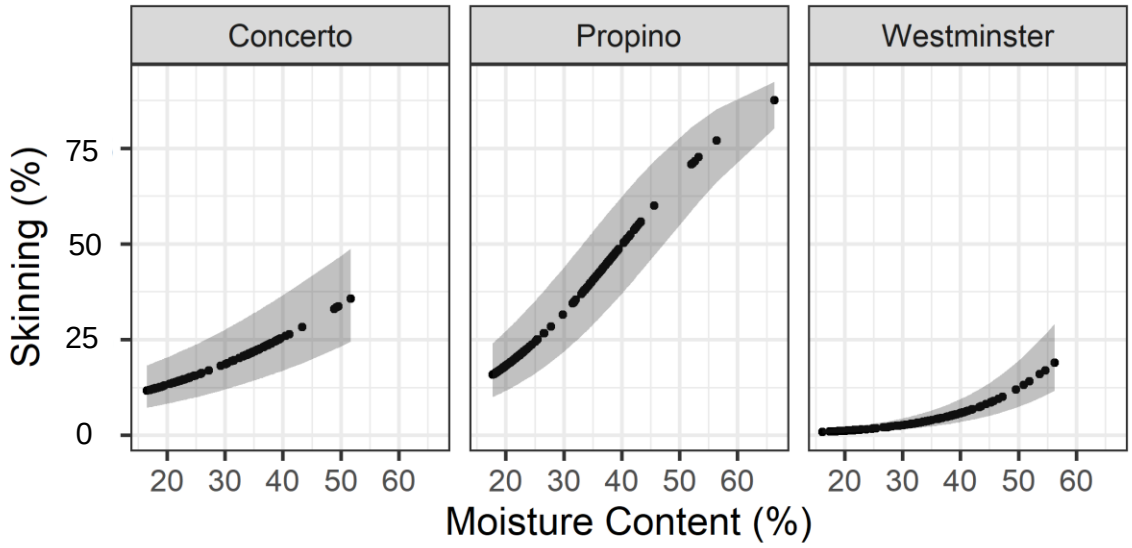


Figure 2.6. Influence of the moisture content associated with environmental moisture in barley grains grown in the field; grey ribbon represents 95% confidence intervals. Field MC

2.3.5 Skinning mechanism

The aim of the light microscopy was to examine skinned grains from two different environments to establish which tissue separated in grain skinning, and whether the same tissues were affected between the two environments. Figure 2.7 shows images of the intact grain (A), skinned grain with damaged parenchyma cells (B), which are

clearly broken, while the cementing layer is visible and intact and grain in which damage of the cementing layer occurred (C) and the remnants of the cementing layer are visible on the surface of the parenchyma, with red arrow pointing to them. The damage of the grains observed under the light microscope was classified according to the skinning mechanism and the results are presented in Figure 2.8. Grains grown in the glasshouse had most commonly lost the husk due to the damage of parenchyma cells of the husk (81% of the grains). The rest of the grains examined were damaged along the pericarp, with no grains damaged along the cementing layer or testa. Grains grown in the field showed only 2.5% of the damage occurred along the parenchyma cells. In the field, most common skinning mechanism was along the cementing layer (79.5%), the rest of the grains, in low proportions skinned along the pericarp (10%) and testa (8%). The skinning mechanism in grains grown in the glasshouse was significantly different to the skinning mechanism occurring in the field (P <0.001)

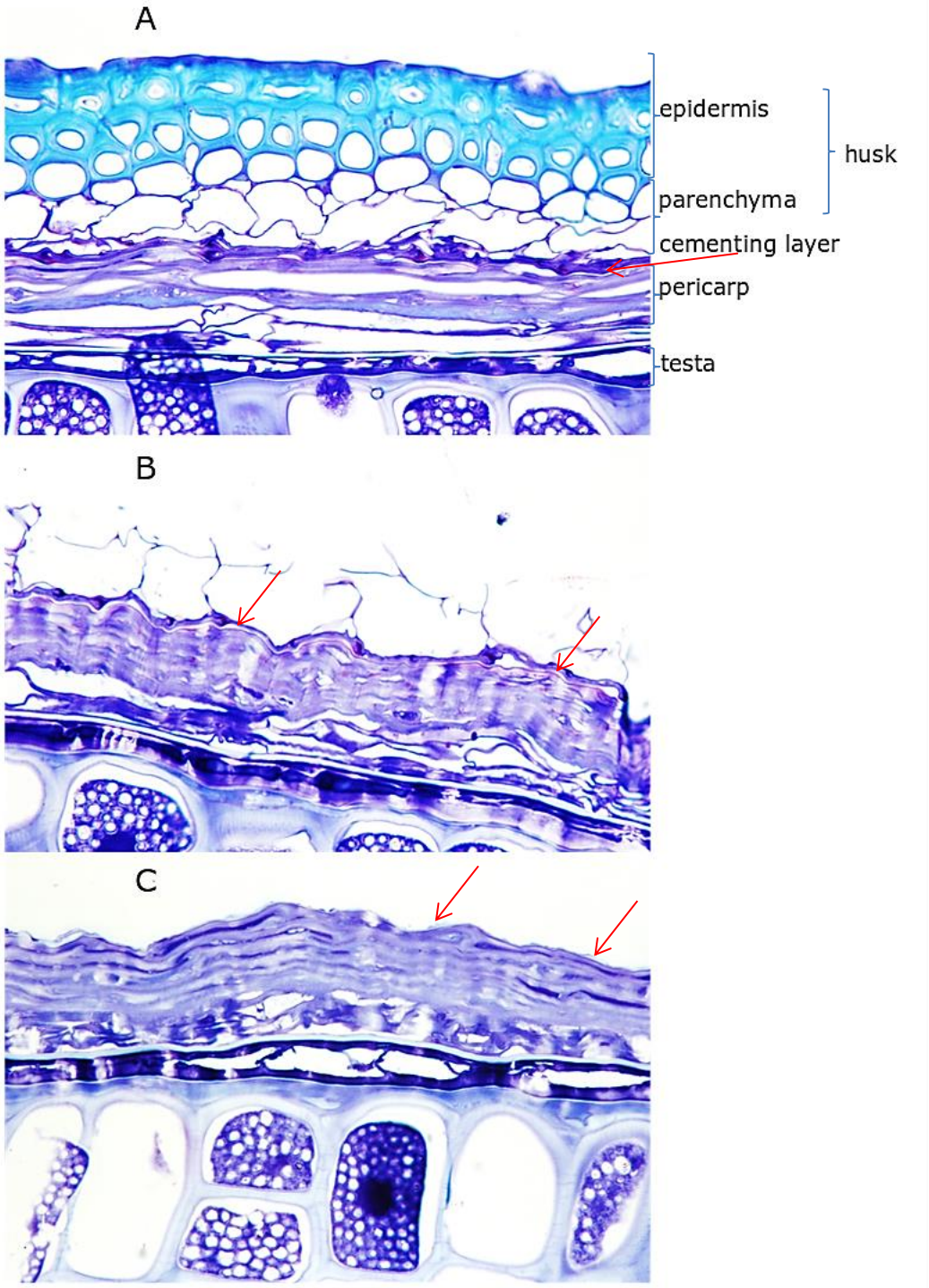


Figure 2.7. Light microscopy of Propino; A. intact grain, B. Skinned grain with damaged parenchyma cells, C. Skinned grain with affected cementing layer. Red arrows in A and B point to the cementilg layer and in C to the visible reminants of cementing layer.

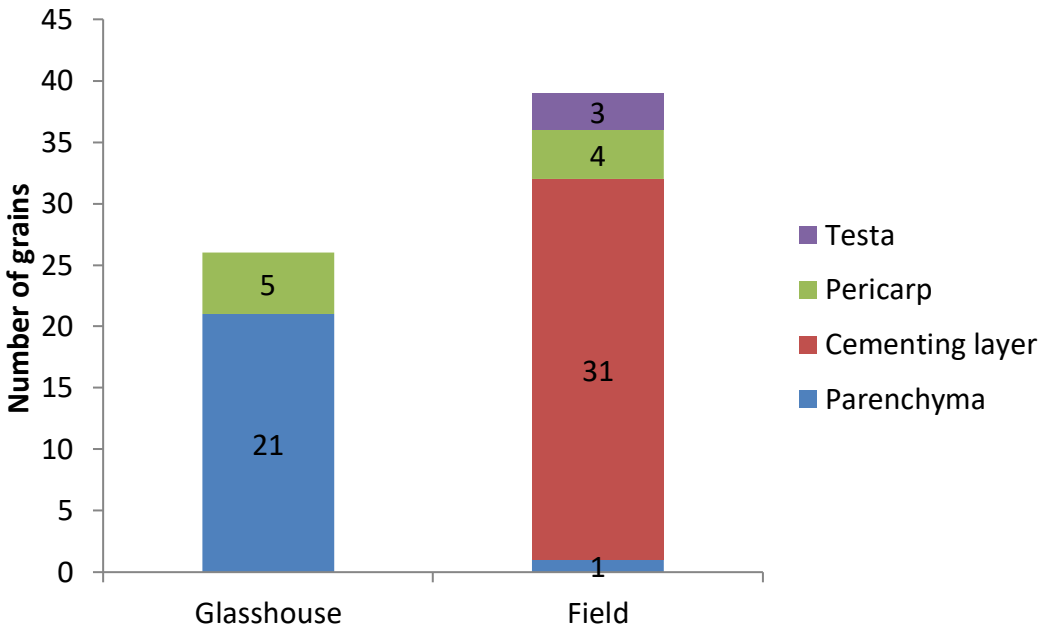


Figure 2.8. Skinning mechanisms in grains grown in two different environments (glasshouse and field) in single variety Propino.

2.4 Discussion

Severity of skinning is dependent on a complex interaction of genetic factors, weather conditions during growing season and at harvest as well as the settings on the combine harvester. Unfortunately most of these factors are well beyond farmers' control, as even choosing the best variety with low skinning susceptibility, does not guarantee it. In addition they are limited in their choice of variety to those approved by AHDB for brewing and distilling. Choosing the right variety and correctly setting the combine harvesters can help farmers minimise the risks of skinning. At present there are no formal guidelines for setting of the combine harvesters, as they range in designs and specifications, leaving a potential trade-off between quality and speed,

up to the subjective choice of the farmer. Other cereals have also been negatively affected by the use of high drum speeds, in wheat and oats it has resulted in in reduced vigour of the grains (Bourgeois et al., 1996, Kirkkari & Rita, 2001). In wheat, loss of vigour was observed in grains with visually intact external tissues, this was attributed to internal damage due to high impact force (Bourgeois, 1993), which is something that is likely to contribute to the mechanisms of grain skinning demonstrated here. The experimental work in this chapter attempted to explain the role of moisture content in the severity of skinning especially in the days either leading to harvest, or during harvest. This would provide data useful for aiding farmers in their decision making process when it comes to choosing the right conditions for harvest.

The first novel finding from this work is the two contrasting roles of moisture content on the severity of skinning: protective and damaging. The protective role was observed in the Glasshouse MC Experiment and Barley soaking experiment (MC's approximately 11%-35% (wb)); and the damaging role was observed in the Misting experiment (MC's approximately 12% - 20%) and Field Moisture Content experiments (MC's approximately 18% - 60%). This difference could be a result of hydration of different tissues of the grain. Cozzolino et al. (2015) observed that different tissues o the barley grain; including the husk and endosperm have different rates of water uptake. Water distribution in the grain might be of more importance than the total moisture content of the grains; unfortunately this was not investigated in this thesis. Total grain MC does not indicate which tissue is hydrated, and it is the hydration of the dry cell walls of the husk that most likely makes them elastic, reducing skinning

(Witrowa-Rajchert & Turek, 1998). Dry cells are more prone to damage and breaking (Lewicki & Pawlak, 2003). It is expected that small quantities of moisture would improve the skinning susceptibility in those grains which were dry, as observed in Barley Soaking experiment. The length of exposure to water was not enough to hydrate the grain, and change the total MC but it could have be enough to hydrate the husk (Cozzolino et al., 2014, 2013; Briggs, 1998). This improved elasticity of the parenchyma cell walls of the husk and reduced the severity of skinning. In Glasshouse Moisture experiment, the cell walls of the grains were not fully dry, retaining the elasticity of the fresh cells. The results of the Glasshouse Moisture experiment suggest that harvesting the grains a few days early, before they are completely dry and possibly before they are exposed to the damaging cycle of wetting and drying could protect the grains from the skinning.

In the experiment investigating skinning mechanism a second novel observation was made, which confirms and further explains the above contrasting findings on the grain moisture. Grains grown in the glasshouse have skinned through breakage of the parenchyma cells of the husk in over 90% of the grains examined. This 'parenchyma cells' mechanism of skinning was previously described by Olkku et al. (2005), but it was not related to the growing environment, method of harvest or moisture content. Parenchyma cells are large, thin walled cells, which hold water (Lewicki, 1998) and are photosynthetic in the green grains (Briggs, 1978). As this cells dry out, they shrink and become more rigid, losing their flexibility and resistance to mechanical stress (Lewicki & Drzewucka, 1998), therefore they are often the cells responsible for skinning in dry grains. On the other hand barley grains grown in the

field, especially in Scotland are exposed to almost daily rain and high humidity levels and those grains have much higher MC at harvest. In those grains damage along the cementing layer, where the husk tissues detach cleanly from the pericarp of the caryopsis, was almost exclusively the cause of skinning. This is in agreement with the theory of weakened adhesion, and 'cementing layer' skinning mechanism was previously proposed by Gaines et al. (1985) and Hoad et al. (2016). The cementing layer is cuticular in origin and it was previously shown in other fruits including tomatoes (Matas et al., 2005) and cherry (Knoche & Peschel, 2006) that exposure of the outer cuticular layer to excessive water causes microcracking of the cuticles and can eventually lead to splitting of the pericarp.

In this project grains grown in the glasshouse were not exposed to hydration during growth and development as the plants were watered directly to the pot. This meant that the grains on the plant were exposed to a slow drying process, and dry grains express the 'parenchyma breakage' skinning mechanism described above. Considering that cereals are fruits, the expected tissue and cell damage upon drying would be comparable to that seen in other fruits. As cell walls dry they become rigid and are easier to break with mechanical force (Lewicki, 1998). Drying changes the plant cell walls from being elastic-visco-plastic to fragile. Szymanska 1975 in Lewicki & Jakubczyk (2004) has attributed this changes to damage of the internal structures of the cell, the cell plasmolysis and denaturation of the biopolymers. Drying causes the plant material to shrink and develop tensions between cell walls. Lewicki & Pawlak (2003) showed that during drying, internal tensions develop when the cells shrink, and that those structures are damaged and deformed. When the water is

removed from the plant material cell walls become more rigid, losing their elasticity while simultaneously building moisture gradients within the plant tissues, resulting in shrinkage stress. This in turn results in fractures, breaks, discontinuities and loosening of the structure of the material, making it more vulnerable to mechanical damage. In addition Witrowa-Rajchert & Turek (1998) reported that it is the shrinkage of the plant material that is most responsible for the change in mechanical properties of the plant material. In the glasshouse moisture experiments these changes did not yet take place in the barley grains, which were not fully ripe and had internal moisture, and therefore the structure of the cell wall have not yet been weakened and remained elastic. Upon rehydration of the dry plant material water plasticises the glassy matrix and structural mobility of polymers increases (Lewicki, 1998). This increase in plasticity of the matrix could explain the protective role of small quantities of moisture absorbed during short soaking in the experiment with barley soaking.

Increased skinning was observed in the experiments where barley grains were exposed to the water for long periods of time. Rathjen et al. (2009) used Magnetic Resonance Micro-Imaging on wheat grains, and barley would be expected to behave in a very similar way, to show that water is first imbibed by the grains into the outermost tissues, and through the micropyle into the embryo of the grains, it then start to move into the endosperm after longer exposure. There was no evidence of water diffusion through the testa, and Cozzolino et al. (2015) confirmed that barley husk and endosperm imbibe water differently. The design of the experiments with barley misting and field moisture content exposed the grains to water for a long time.

When the water starts moving into the grain it is likely that the cementing layer is affected by moisture in the same way other adhesives in material sciences are: water permeates the adhesive and displaces it at the bonding site (Ebnesajjad & Landrock, 2009). In addition, misting experiments conducted by Froment & South (2003) and by Brennan et al. (2017b) both showed increase in skinning severities of treated grains, even though the two experiments were conducted in different environments: field and glasshouse, respectively.

Overall, the experimental work presented in this chapter covers a range of moisture content from 10% - 45%. The results of the experiments indicate that the best moisture content of the grains for harvest falls between 15% and approximately 22%. Levels of grain moisture content below 15% would likely result in skinning due to dryness and breakage of parenchyma cells. This scenario is not likely to happen in Scotland, where humidity and rainfall are high. Dry conditions are more likely to cause skinning in the south of UK and other European countries, where rainfall during growing season is lower than in Scotland. Grains harvested with levels of moisture above 22%, the scenario most likely responsible for skinning in Scotland, results in damage and displacement of the adhesive layer between the husk and caryopsis.

The understanding of cell wall polymers in other plants can further help us understand these two different responses to moisture in barley grains. Cell walls of wood, when exposed to repeated drying and rehydration showed changes in structure, specifically a separation of the cellulose microfibrils and matrix substances (Toba et al., 2012), drying of the wood changed the width of the cellulose crystals in

cell walls of wood (Fang & Catchmark, 2014). In studies on onion cell wall polymers, dry cell walls behaved as rigid solids and authors suggested that the theory of composite materials could apply. Therefore when the cells were rehydrated, the cellulose/xyloglucan microfibrils behaved as solid rods, and the largest physical change within the hydrated cell wall occurred between the microfibrils and the matrix, composed mostly of pectin (Ha et al., 1997) and water is critical for the flow-like behaviour of cell wall matrix polymers (Zamil et al., 2015). The behaviour of cell wall polymers help to further explain the findings of this project. Dry cell walls are rigid and can easily be broken as seen in glasshouse grown barley, rehydration of the cells causes the matrix to become more fluid-like, offering protection from mechanical damage, to the dry and rigid cells of the glasshouse grown barley.

This information is not just scientifically novel, but most importantly has a practical application in aiding the farmers in their decision making on the best time to harvest grains to prevent or at least reduce the skinning in the grains they are harvesting. Only grain bulks with skinning below the threshold set by the maltsters will be accepted into malting and the farmer will receive the premium price, if skinning exceeds this levels the grains will be sold for feed, with a loss to the farmer. Development of precise guidelines for farmers would require more research to accurately indicate the time of exposure and moisture levels to minimise skinning severities, however the results of this project encourage the farmer to consider the moisture content and the weather before commencing the harvest.

2.5 Conclusions

This chapter aimed at investigating the influence of moisture on severity of skinning in malting barley. The novel findings of this project were that moisture has two contradictory roles in skinning: Dry grains are susceptible to skinning, which most commonly occurs by “parenchyma cells” skinning mechanism. Therefore, grains which are very dry benefit from a little bit of moisture, which increases the elasticity of the cell walls. However prolonged exposure to water causes the cementing layer to become displaced by water and adhesion is weakened, and those grain express “cementing layer” skinning mechanism. This type of skinning is characterised by clean detachment of the husk from the caryopsis at the cementing layer. The perfect harvest moisture content of barley grains is between 15% and 22%, based on the results of this work. This could be developed further into formal guidelines for farmers, helping in their decision making on the best harvest conditions.

Chapter 3. Assessment of the quality of malt produced from skinned grains

3.1 Introduction

Previously, chapter 2 explained how in the weeks before harvest barley moisture affected skinning, and how harvesting grains which have been exposed to excessive moisture is likely to reduce their quality for malting by causing skinning, an undesirable form of grain damage. Chapter 3 quantifies how grain samples with different levels of skinning affect characteristics of barley grain quality, and subsequently the malting process and final malt quality.

Skinning has become increasingly prominent in modern barley varieties, and almost all modern malting variates are prone to this defect (Brennan et al., 2017b). This condition causes uneven malt modification, which in turn is problematic for the malting industry, and results in lower efficiency of the whole process (Okoro et al., 2017). Improving the efficiency of the malting process allows for increased recovery of the sugary extract from the same quantity of grains, and therefore cost-effective production of the malt, which is beneficial for the whole supply chain including growers, maltsters, brewers and distillers.

Malting is a process of controlled germination, during which a breakdown of cell walls of starch granules takes place and starch is released making it available for

further breakdown during brewing or distilling, this process is described in detail in Chapter 1 (Introduction).

The first and most important role of the husk in malting is protection of the embryo during harvest, transport and malting, as only grains with intact embryos are able to germinate and modify (Roumeliotis et al., 2013). Skinning potentially affects malting at each of the malting steps: steeping, germination and kilning. The understanding of the effects of skinning on malt comes mainly from anecdotal and historical evidence (Meredith, 1959). My study is the first one to attempt to quantify the effects of skinning on malt quality in modern barley varieties. During steeping skinned grains take up water faster than grains with intact husks (Agu et al., 2009; Swanston & Middlefell-Williams, 2012) causing one of two possible situations: the grains germinate faster and overmodify, or the excess water drowns the embryos, resulting in lack of germination. After steeping, grains germinate usually for four days, during which the husk protects the growing acrospire, which grows along the grain, under the husk. Grains in which acrospires have been damaged or knocked off will not modify fully. The final stage of malting is kilning, where the husk offers protection to the grain, however this time it protects the modified and friable endosperm, creating a 'package' and preventing the endosperm from being easily damaged. Damaged endosperm in malt increases the particulate matter or dust (Olkku et al., 2005), which needs to be carefully extracted in the brewery or distillery, to avoid health risks to the employees, and this in turn increases the overall processing costs. Husks play an important role through the malting process, and good quality, uniform in size and composition barley grains with low levels of skinning result in

homogeneous malt. This in turn allows for maximum modification and therefore maximum sugar recovery in later processes. Although it is possible to produce good quality malt from naked barley (Agu et al., 2008, 2009; Swanston et al., 2011; Swanston & Middlefell-Williams, 2012; Edney & Langrell, 2004), which is similar in appearance to barley grains that are completely skinned, currently malting facilities are set up to process covered grain only. A crucial issue with barley skinning is that it is likely to affect only a proportion of the grains in the bulk. This variation has consequences for the malting industry as it relies on homogeneity in grain quality throughout the process. It starts with the barley grain samples, through the germination phase to the final malt. Uniformity is one of the key requirements to ensure efficiency in the whole process.

The malting industry uses standard methods to evaluate the quality of malt produced. In the UK the methods most commonly used are Institute of Brewing Methods of Analysis, which have been incorporated into the European Brewing Convention Analytica in 1997. These methods are used to evaluate barley grains and the quality of malt produced. The process of evaluation starts with grain analysis and measurement of thousand corn weight (TCW) and specific weight (SW) of the grain bulk. High TCW and SW signify large, plump grains desired by the maltsters, although the factors influencing SW are not yet very well understood and they are influenced by interaction of complex factors including grain size and density (Hoyle et al., 2018).

Currently in the UK, hot water extract (HWE) is the most common method of evaluating malt quality and it is routinely used in selecting new varieties for the

recommended list, in breweries for purchasing of the malt and for research purposes. Hot water extract is a measure of the simple sugars extracted from malted barley grains dissolved in the solution. It is a process simulating the mashing in brewing or distilling, where small samples of malt are mashed with water and heated to the optimal temperature (~70^{o}C) for hydrolytic enzymes to break down the starch. The sugars in the extract, which in brewing is also called wort, are obtained from the mash then measured using density meters. The HWE is calculated using those densities and expressed as l^{o}/kg. Volume of the extract collected in 30 minutes is an additional measure of malt quality, undermodified grains have a lot of β-glucan and storage proteins, which slow down filtration and results in a cloudy filtrate. Although this method is not very reliable for comparison between varieties or even harvest seasons, in this Chapter it has been useful for comparison between the skinning categories of the same variety. This small scale, analytical mashing method does not achieve the same concentrations of sugars the brewery would get from the same malt; however this method has been used for a long time and HWE results are easily translated into the performance of the malt in the brewery.

Good quality malt has high HWE as described above but should also be evenly modified meaning that none of the modifiable endosperm is “wasted”, i.e. it is all made available for enzymes during the mashing process. Hydrolytic enzymes are responsible for the starch breakdown and measuring their levels in malt informs of the quality of malt produced. Most abundant enzymes are α- and β- α-amylases as described in detail in Chapter 1. In this study I have measured α-amylase, because it is synthesized *de novo* during germination, unlike β-amylase, which is always present

in the grain and its levels would not be expected to be affected by absence of the husk. High levels of these enzymes are indicative of good quality malt, capable of rapid and even modification.

Friability and homogeneity are physical measures of the modification of the malt. Breakdown of the cell walls surrounding starch granules during germination step makes the grains friable and easy to crush, and it is a measurement of the proportion of the grain in the samples which are easily crushed. One of the most desirable traits of good quality malt is homogeneity – all the grains need to germinate evenly and achieve full modification at the same time, if this did not happen malt would be a mixture of undermodified and over modified grains. In this Chapter I present data on friability and homogeneity on the bulk of the malt, however it must be remembered that the friabilimeter method tends to overestimate the modification in certain circumstances of malt (Darlington & Palmer, 1996); investigation into modification of the malt in much greater detail is described in Chapter 4. During germination, growth of the roots and acrospire takes place and if excessive growth occurs, this indicates overmodification, as the plant is using the sugar for its development and the growth should be stopped by kilning the malt. Loss of the weight of the grains due to the respiration and root and acrospire growth is termed 'malting losses' and excessive malting losses are indicative of overmodification.

Methods selected for malt quality analysis in this Chapter are standard, well-recognised methods selected from a large number of possible tests and they give a

good insight into the malt quality and allow for comparison between the skinning severities of barley samples. The methods were selected based on their increased likelihood of being affected by the loss of husk, and some methods routinely used to assess malt quality, including various measurements of protein and nitrogen in the grain, were not used.

3.1.1 Aims and objectives

Understanding of the impact of skinning on the malting process and malt quality is based mostly on anecdotal and historical evidence. The aim of this chapter was to quantify the effects of skinning on the malting performance of two modern malting varieties. The objectives were: i) to establish a test set of four distinct categories of skinning severity in two barley varieties; ii) to quantity effects of skinning on the quality of the barley sample and malt quality measures, including HWE and iii) to develop a procedure for adjusting HWE that accounts for loss of husk biomass.

3.2 Materials and methods

3.2.1 Grain samples

Two spring barley (*Hordeum vulgare*) varieties, Concerto and Chronicle were used for grain skinning and malting tests. Both varieties have been in commercial use and

listed as brewing and distilling varieties on the AHDB Recommended Lists. Although similar in their performance under standard micro-malting tests, Chronicle was less consistent than Concerto in macro-scale and commercial bulks and was removed from the Recommended List in 2014. For both varieties, a main stock of 20 kg was sourced from Bairds Malt in Arbroath at 2015 harvest intake and from Bairds Malt in Pencaitland at 2016 harvest intake. Skinning in both varieties was previously described by Brennan et al., (2016) and expected skinning levels for chronicle were 9.2% - 16.6% and for concerto 16.4%. Each stock was scored for grain skinning to establish its overall field level of skinning (see below).

3.2.2 Skinning assessment

All samples were screened over a 2.5 mm sieve to remove small grains prior to assessment. Grain skinning was scored using a protocol developed by SRUC and the Institute of Brewing and Distilling (Scottish Micromalting Group, The Maltsters' Association of Great Britain, Nottinghamshire, UK). Each grain was examined on both the ventral and dorsal side and grains with overall husk-loss exceeding 1/5 of the total surface area were scored as skinned.

3.2.3 Skinning categories

After screening, four 1.5 kg bulks corresponding to different proportions of skinned grain, were created from the Concerto and Chronicle stocks. The bulks were manipulated to create four skinning categories: 'intact' grains with less than 1% skinning, 'mild' created from untreated field stocks with a range among samples of 14.8% to 18%, 'skinned' with a range from 49.9% to 51.8% and 'severe' from 84.5% to 91.8% (Table 3.1). The intact category was created by visually assessing small samples of approximately 30 g of grains from the main Concerto and Chronicle stocks and removing any grains that were skinned, until a bulk of 1.5 kg was achieved. Mild category was sampled directly from the screened, field level stocks. The skinned category was achieved by threshing stock grain using a Wintersteiger LD 180 (Wintersteiger, Reid, Austria) laboratory thresher at the speed setting 1 for 10 s, in samples of 200 g each time. The severe category was achieved by threshing samples for 10 s with the thresher speed setting 5.

Table 3.1. Skinning Categories and mean levels of skinned grains in the bulk ± standard deviation.

Year	Variety	Category	Skinning (%) ± SD
2015	Concerto	Intact	<1.0
		Mild	14.8 ± 3.6
		Skinned	49.9 ± 4.6
		Severe	84.5 ± 3.5
	Chronicle	Intact	<1.0
		Mild	18.9 ± 3.4
		Skinned	51.8 ± 2.7
		Severe	91.8 ± 3.2
2016	Concerto	Intact	<1.0
		Mild	18.5 ± 3.2
		Skinned	49.3 ± 4.3
		Severe	90.7 ± 4.0
	Chronicle	Intact	<1.0
		Mild	18.0 ± 2.1
		Skinned	51.0 ± 3.6
		Severe	88.7 ± 1.9

3.2.4 Grain analysis

3.2.4.1 Thousand Corn Weight (TCW) and Specific Weight (SW)

Assessments of TCW and specific weight, in triplicate, for each skinned bulk were conducted at SRUC's Boghall Farm near Edinburgh. A Numigral grain counter (Tecator Ltd., Sweden) was used to count a sample of 1000 grains and record its weight. Specific weight of the bulk grain samples was measured using a Dickey-John (GAC2000, Dickey-John Corporation, UK).

3.2.4.2 Moisture Content

To measure moisture content, the ASAE S35.2 method was modified to reduce the required sample size of unground grain from 10 g to 1 g. A bulk of Concerto grains was used to determine whether the modified method gave the same results as the original. The difference in moisture contents between large and small sample sizes was not statistically significant. In the final method, 1 g of unground grain was placed in aluminium weighing dishes and dried at 130°C for 20 h. The dishes were cooled in a desiccator over indicating silica gel, and then weighed (Sartorius GMBH, Göttingen, Germany, accuracy: 0.0001g). This was carried out in triplicate. The percentage of moisture (wet basis) was calculated by dividing the loss in weight by the weight of the original sample and multiplying by 100.

3.2.4.3 Germinative Capacity (H_2O_2 method)

Standard germination tests, using IoB method 1.5 (IOB, 1997) were performed on sub-samples from each of the skinning categories created in section 1.3 for both varieties to account for any damage caused by the thresher.

Three replicates of 200 grains from each category were incubated in 200 ml of an aqueous solution of 0.75% (v/v) hydrogen peroxide, made up freshly from a 30% (w/v) concentrated H_2O_2 stock solution (Thermo Fisher Scientific, product no 10386643). After two days of incubation grains were drained and fresh solution of 0.75% (v/v) hydrogen peroxide solution was added. Grains were then incubated for another day, totalling three days of germination at 18°C.

After three days the solution was drained and grains which had not chitted (root/acrospires appearing) were separated. Husks were removed on all the corns with no visible chit, to examine for any growth under the husk. Grains with no chit were counted and the percentage of chitted corns was calculated and is reported as germinative capacity (%).

3.2.4.4 Germinative energy (4 ml test) and water sensitivity (8ml)

The germinative energy test indicates the percentage of grains capable of germinating in normal conditions. Both the 4 ml and 8 ml tests were performed using a standard IoB method (IOB, 1997). Two filter papers were placed in the bottom of a 9 cm (diameter) plastic petri dish, to which 100 grains were added followed by addition of 4 ml of tap water for germinative energy and 8 ml for the water sensitivity test. Grains were germinated at 18^{o}C, for 72 h in the dark, and the number of grains with visible roots/acrospires was recorded as percentage of total grains

3.2.5 Micromalting

The micromalting station used at Heriot-Watt is shown in Figure 3.1A. All the samples were run in triplicate, with samples of each replicate type placed within a different bin position and unit position among runs (Figure 3.1B) to avoid positional effects. The program used is routinely used for malting standard, non-water sensitive barleys (Martin & Bamforth, 1980; Agu et al., 2008). The program consists of a 48 h steeping

regime: 8 h steep, 16 h air rest and 24 h steep, followed by four days of germination, both germination and steeping at 16°C. Samples from the 2015 harvest season were micromalted at Heriot-Watt University, Edinburgh, and samples from the 2016 harvest were micromalted at the Scotch Whiskey Research Institute (SWRI), Edinburgh, due to subsequent technical issues with the micromalting unit at Heriot-Watt. The main difference between using the two units was that the samples micromalted at Heriot-Watt were mixed manually, and at SWRI they were mixed automatically every day to prevent the formation of heat-spots and excessive clumping of roots.

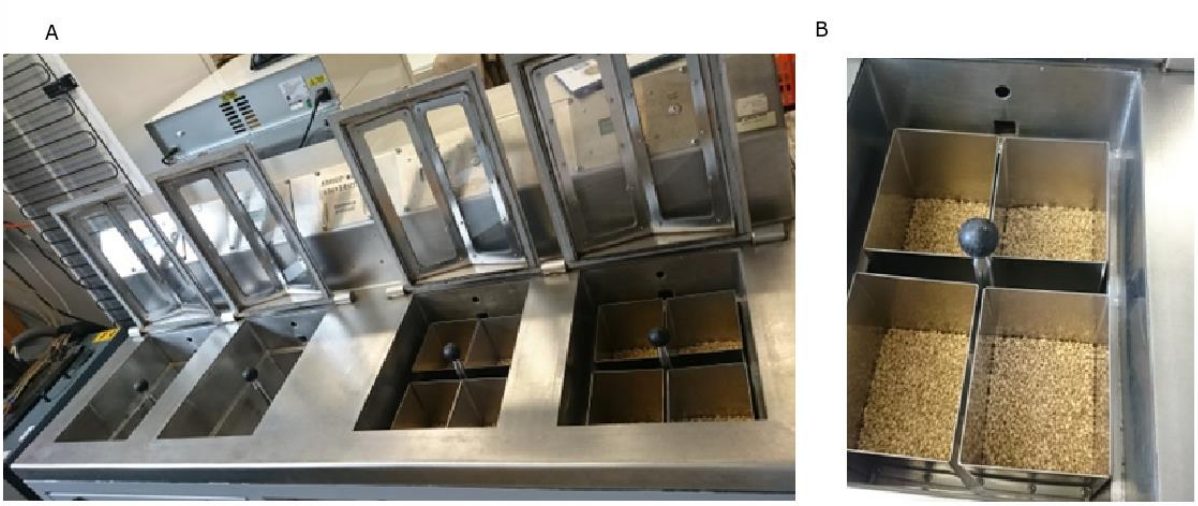


Figure 3.1. A. micromalting station at Heriot-Watt University; B. Each unit consists of four bins of 500g capacity

3.2.5.1 Kilning

After germination, the samples were transferred to the kiln (Figure 3.2) for 24 h at 50°C, after which the roots and acrospires were knocked off by rubbing the grains by

hand on a 2.25 mm sieve. Both grains and roots were weighed and placed in plastic bags for storage before further analysis; weight was recorded and was used to calculate malting losses.

Figure 3.2. Kiln at Heriot-Watt University

3.2.5.2 Malting losses

The weight of the final malt, weight of the rootlets and moisture content of both barley and malt was adjusted to dry basis (db) and used in the calculation of the total malting losses, which is a weight of the grain lost in germination process due to respiration, root and acrospire growth as well as other losses. High malting losses would indicate overmodified malt or could highlight other issues in processing. Malting loss is calculated using equation:

Equation 1. Malting loss

$$Malting\ Loss = \left(Barley\ weight\ (db) - Malt\ weight(db)\right)x100\ /Barley\ weight(db)$$

3.2.6 Malt quality analysis

3.2.6.1 Hot water extract

The method used in this assessment is an IoB method 2.7 (IOB, 1997). A 50 g sample of malted barley grains were milled with a Bühler-Miag mill, 0.7 mm disc clearing setting (Bühler-Miag mill DLFU, Bühler Ltd, London) (Figure 3.3A), placed in labelled metal cup and inserted into a holder in the water bath (Figure 3.3B) set to 67^{o}C. Cups were filled with 360 ml of distilled water at the same temperature and magnetic stirrers were placed in each of the cups to agitate the mash throughout the process. The mash was held at 67^{o}C for one hour after which it was cooled down to 20^{o}C. When the mash reached this temperature, it was quantitatively transferred into volumetric flasks and the volume was adjusted to 515 ml using distilled water. The contents of the volumetric flask were vigorously shaken and then the whole content was poured onto a funnel lined with filter paper (Figure 3.3C). The first 50 ml of the filtrate was returned to the funnel. This first filtrate is always cloudy, because it does not go through the bed of grain and husk. The density of the final filtrate was measure using the Anton Paar DMA46 (Anton Paar Ltd.; St Albans UK) (Figure 3.3D) density

meter calibrated with distilled water and the density of the extract was used to calculate the HWE in l^{o}/kg using equation:

Equation 2. Hot Water Extract calculation

$$HWE\ (l^{O}/kg) = (((density\ of\ extract/density\ of\ water\ -1) * 1000) * factor$$

Where:

Density of water = 0.9982 g/cm^3

Factor = 10.13

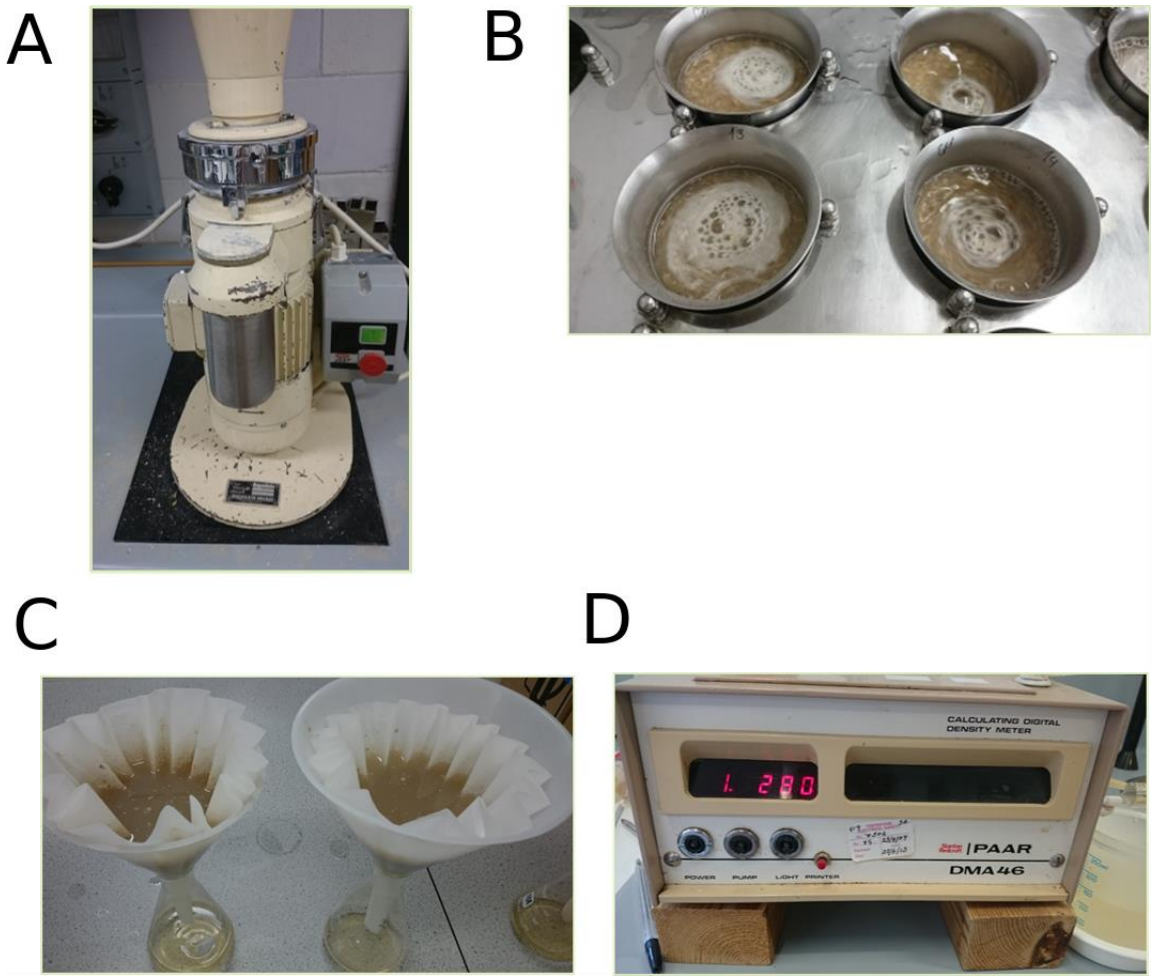


Figure 3.3.Hot water Extract preparation. A-Bühler-Miag mill; B- mashing in of the 50g samples; C- filtering of the samples through the grain bed and a filter; D- Anton Paar density meter

In addition to measuring HWE this method measures the volume of the filtrate collected in the first 30 min of the filtration, starting after the initial 50 ml was returned to the funnel. Volume (ml) can be used as a method in addition to HWE as an indication of malt modification – unmodified malt is high in β-glucan, making it viscous and the filtration is slower.

3.2.6.2 Adjusted Hot Water Extract Calculation

An adjusted HWE (AdjHWE) is a measure developed for this project, based on research by Meredith (1958), who reported their result on per grain basis to account for lost biomass due to skinning, but no calculation method was reported in their paper. AdjHWE was calculated to account for the weight of the husk lost from the 'mild', 'skinned' and 'severely skinned' categories, based on the differences in TCW between those and the 'intact' category. The samples used in HWE measurements use 50 g of the sample; AdjHWE accounts for a number of grains in that sample, and calculates the HWE per number of grains, rather than per 50 g, and adjusts this to include the weight of the lost husk for that category (based on the TCW difference). All the calculations on the malt and barley were performed after adjusting the analysis to dry weight. Adjusted HWE was calculated using the formula:

Equation 3. Calculation of AdjHWE

$$AdjHWE\ (l^{O}/kg)\ = \left(HWE/GNo_{adj}\right)\ *\ GNo_{i}$$

where HWE is the hot water extract (l°/kg) from a 50 g sample of malt, GNo_i is the intact number of grains in the 50 g sample of malt, and GNo_{adj} is the number of grains adjusted for weight of husk-loss in the 50 g sample of malt as calculated below:

Equation 4. Calculation of grain number in intact and adjusted categories

$$GNo_i = dSampleI_n/[dTCWi/1000]$$

$$GNo_{adj} = dSampleX_n/[dTCWX/1000]$$

and $dSampleI_n$ is the dry weight of the nth subsample of each of the intact (I) category samples, $dSampleXn$ is the dry weight of the nth subsample of each of the X skinning categories, $dTCW_i$ is the dry thousand corn weight of the intact samples, and $dTCW_X$ is the dry thousand corn weight for each of the X skinning categories.

3.2.6.3 Friability

Friability was measured using friabilimeter (Pfeuffer GmbH, Kitzingen, Germany). A 50 g sample of barley malt was placed in the rotating sieve of the friabilimeter (Figure 3.4). The meter was set to rotate for 8 min and a roller inside the round sieve crushes the grains. Malted and modified grains crush easily and finely, and the friable flour falls through the sieve onto the tray. The grains which were not modified were hard and non-friable and were therefore retained by the sieve. This portion was then weighed, and the friability score was calculated using the equation:

Equation 5. Friability

$$Friability(\%) = 2 * \left(50\ g\ -\ Weight\ of\ Nonfriable\ Part(g)\right)$$

Figure 3.4. Friabilimeter at Heriot-Watt University

3.2.6.4 Homogeneity

Homogeneity of the malt modification was calculated by passing the non-friable portion of the grains from friability assessment through a 2.2 mm sieve. The proportion retained on the sieve was weighed and homogeneity of the sample was calculated using the equation:

Equation 6. Homogeneity

$$Homogeneity\ (\%) = 2 * \left(50g - weight\ retained\ on\ 2.2\ mm\ sieve(g)\right)$$

The number of whole grains in the non-friable portion retained on the sieve after the homogeneity test was also recorded.

3.2.6.5 Malt α-amylase

A Megazyme α-Amylase Assay Kit (Ceralpha Method) (Megazyme, Ireland) was used to assess the α-amylase content of the Chronicle malts from harvest season 2015 and 2016. The assays were done under my supervision by Eilidh Wood (Wood, 2018), a BSc dissertation student whom I supervised during my PhD. The kit included concentrated reagents and instructions on preparation of reagents not included in the kit, or additional concentrated reagents. Reagents that were supplied in the kit were the following: amylase HR reagent, extraction buffer and stopping reagent. The other reagents were prepared according to the kit instructions as detailed here. The malt extraction solution (1 litre) was made with 1% (w/v) sodium chloride, 0.02% (w/v) calcium chloride and 0.02% (w/v) sodium azide in distilled water. Concentrated Extraction Buffer (1 litre) was prepared by dissolving Malic acid 134.1 grams/litre, Sodium hydroxide 70 grams/litre, Sodium chloride 58.4 grams/litre, were added to 800 ml of distilled water, this was allowed to cool to room temperature, and Calcium chloride dihydrate (40 mM) 5.9 grams/litre was added. After adjusting pH to 5.4 sodium azide (Sigma S2002; 0.1%) 1.0 grams/litre was added and volume adjusted with distilled water to 1 litre. For use, 50 ml of this concentrated buffer was diluted to 1 litre with distilled water. The additional buffer was prepared by dissolving tri-

sodium phosphate (anhydrous) (10 g) in 1 litre of distilled water and adjusting the pH to 11.0.

Malt α-amylase extract

Chronicle malt from the micromalting experiments above was used in the assessment of malt α-amylase. Malt samples (20 g) were milled using IKA A11 B grain mill (IKA, England LTD, Oxford, UK) to a very fine flour and 0.5 g samples were weighed out into a 100 ml volumetric flask. Malt Extraction Solution was added to flasks and made up to 100 ml volume with distilled water. The flasks were left at room temperature for 15 min with occasional stirring, to allow for the enzyme to be extracted. Aliquots of the solution were centrifuged at 1,000 g for 10 min. The supernatant (0.5 ml) was diluted with 9.5 ml of Extraction Buffer Solution and the samples were frozen for no longer than two weeks.

Enzyme activity assay

Malt α-amylase samples were thawed, and enzyme activity was assessed. Amylase HR Reagent aliquots (0.2 ml) and malt extract solutions (0.2 ml) were dispensed into test tubes and pre-incubated at 40°C for 5 min. To each tube containing Amylase HR Reagent solution 0.2 ml of malt extract was added directly to the bottom of the tube. This was incubated at 40°C for exactly 10 min (from time of addition). At the end of the 10 min incubation period, 3.0 ml of Stopping Reagent was added, and the tube contents were stirred vigorously. The absorbance of the solutions was read using a spectrophotometer at 400 nm (Agilent 8453 UV-visible Spectroscopy System, Agilent Technologies, Santa Clara, California USA) against the blank, which was created by

adding the Amylase HR Reagent aliquots (0.2 ml) and malt extract solutions (0.2 ml) directly to the 3 ml of stopping reagent without allowing for any development time.

Calculation

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one micromole of *p*-nitrophenol from BPNPG7 in one minute under the defined assay conditions, and is termed a Ceralpha Unit (McCleary et al., 2002; McCleary & Sheehan, 1987).

Units/g Flour:

Equation 7. Calculation of Ceralpha Units

$$\frac{\Delta E_{400}}{Incubation\ Time} x \frac{Total\ Volume \in Cell}{Aliquot\ Assayed} x \frac{1}{\varepsilon_{mM}} x \frac{Extraction\ Vol}{Sample\ weight} x Dilution$$

where:

ΔE_{400} – Absorbance (reaction) - Absorbance (blank)
Incubation Time – 10 min
Total Volume in Cell – 3.4 ml
Aliquot Assayed – 0.2 ml
$^{\varepsilon}$mM of *p*-nitrophenol (at 400 nm) in 1% tri-sodium phosphate – 18.1
Extraction volume – 100 ml per 0.5 gram
Dilution – Dilution of the original extract (= 20-fold for malt extracts)
Sample weight – 0.5g

For malt this is:

Equation 8. Calculation of Ceralpha Units in malt

$$\frac{\Delta E_{400}}{10} x \frac{3.4}{0.2} x \frac{1}{18.1} x \frac{100}{0.5} x 20 = \Delta E_{400} x 376$$

3.2.7 Statistical analysis

Statistical analyses were conducted using R (R Core Team 2016) and package *lme4* (Bates et al., 2015) to fit linear models. Linear mixed effects models (LME) were used to determine the effects of the variety and category (predictor variables) and their interaction on each of the response variables: HWE, AdjHWE, Friability and α-amylase. The fixed effects were variety and category, and random effects were position, nested within year, sample no and run. The minimally adequate models were chosen by comparing hierarchical models using ANOVA (α = 0.05) and significant differences among the effects were determined by least squares means pairwise comparisons using package 'lsmeans' (Lenth, 2016). For all response variables, the difference between the two varieties was not significant (P <0.05).

3.3 Results

3.3.1 Grain analysis

Thousand corn weight (TCW) and specific weight (SW) were adjusted to dry basis; the mean and standard deviation (SD) of each variety and each harvest season are presented in Table 3.2.

In both seasons, the skinned and severely skinned bulks of Concerto and Chronicle had lower TCW than the mild and intact bulks. Within each variety and season, the intact bulk had the highest TCW compared to the other three categories. This did not correspond with SW, as the intact bulk had the lowest SW compared with the other bulks.

Germinative capacity (under H_2O_2) was very high in the 2015 harvest season for both varieties in the intact, mild and skinned categories, only the severe category had slightly lower germinative capacity in that season. This pattern was not repeated in 2016 with all four categories in Concerto and Chronicle scoring very highly (above 99%). In both varieties and harvest seasons the grains with the most severe skinning in the bulk had the lowest germinative energy (4 ml test), whereas the intact category consistently had the highest scores. The water sensitivity test (8 ml) showed no signs of excessive water sensitivity in any of the categories, with no clear pattern of any category performing better or worse, although it varied between the years and harvest seasons. Mean results of all the germinative tests are presented in Table 3.2.

Table 3.2 Mean data from grain quality analysis of Concerto and Chronicle, harvest seasons 2015 and 2016

Year	Variety	Category	Skinning (%) ± SD	TCW (db) ± SD	SpecW (db) ± SD	Germ. capacity H_2O_2 (%)±SD	Germ. energy 4ml (%)±SD	Water sensitivity 8ml (%)±SD
2015	Concerto	Intact	<1.0	38.7 ± 0.27	55.91 ± 0.13	99.7 ± 0.6	99.7 ± 0.6	86.7 ± 1.5
		Mild	14.8 ± 3.6	38.3 ± 0.13	56.07 ± 0.79	99.3 ± 0.6	97.7 ± 1.5	87.3 ± 1.0
		Skinned	49.9 ± 4.6	36.3 ± 0.44	59.99 ± 0.1	98.7 ± 2.3	99.0 ± 0	87.0 ± 1.0
		Severe	84.5 ± 3.5	36.4 ± 0.45	65.65 ± 0.34	95.5 ± 1.5	96.8 ± 1.5	82.0 ± 0.6
	Chronicle	Intact	<1.0	39 ± 0.18	55.44 ± 0.1	99.3 ± 0.6	100.0 ± 2.9	75.7 ± 15.5
		Mild	18.9 ± 3.4	38 ± 0.24	55.76 ± 0.56	99.3 ± 1.2	98.7 ± 1.5	69.7 ± 19.6
		Skinned	51.8 ± 2.7	36.2 ± 0.32	61.97 ± 0.15	99.3 ± 1.2	100.0 ± 0	88.3 ± 1.15
		Severe	91.8 ± 3.2	36.1 ± 0.34	66.13 ± 0.47	95.7 ± 1.5	96.0 ± 2.1	78.0 ± 2.0
2016	Concerto	Intact	<1.0	41 ± 0.23	58.4 ± 0.5	99.3 ± 0.8	99.3 ± 0.5	75.3 ± 4.0
		Mild	18.5 ± 3.2	40.2 ± 0.15	58.6 ± 0.23	99.5 ± 0.5	98.7 ± 0.5	80.7 ± 0.9
		Skinned	49.3 ± 4.3	39.3 ± 0.13	62.6 ± 0.15	99.5 ± 0.5	98.0 ± 1.4	86.3 ± 2.1
		Severe	90.7 ± 4.0	39.6 ± 0.13	64.2 ± 0.09	99.8 ± 0.3	95.0 ± 0.8	77.0 ± 1.4
	Chronicle	Intact	<1.0	39.3 ± 0.17	56.4 ± 0.13	99.3 ± 0.3	98.7 ± 1.2	73.7 ± 0.5
		Mild	18.0 ± 2.1	38.1 ± 0.18	56.1 ± 0.31	99.8 ± 0.3	97.3 ± 0.5	77.0 ± 4.3
		Skinned	51.0 ± 3.6	37.4 ± 0.25	60.9 ± 0.22	98.5 ± 0.5	97.3 ± 1.7	79.5 ± 1.5
		Severe	88.7 ± 1.9	37.5 ± 0.31	64 ± 0.37	100.0 ± 0.0	95.7 ± 1.2	78.3 ± 3.1

3.3.2 Micromalting

Micromalting took place in two different units: in 2015 the samples were micromalted at Heriot-Watt and in 2016 at the Scotch Whisky Research Institute, due to subsequent technical problems with the Heriot-Watt micromalting unit. Even though the malting program used was the same for both years, there were differences in processing between the two years, especially in the severely skinned category, which in 2015 did not modify fully and was infected with fungal and bacterial growth, especially replicates two and three. This lack of modification was evidenced by a very limited rate of root and acrospire growth and very high microbial infection rate (Figure 3.5).

A

B

Figure 3.5. Grains of Concerto during micromalting, germination day 4. A. Intact category B. skinned, with visible differences in root and acrospire growth.

3.3.2.1 Malt quality as influenced by year, variety and skinning severity

The malt produced in both years was of a good quality, as determined by good friability, homogeneity and HWE (Table 3.3). Friability was very high for both years and both varieties in the intact, mild and skinned categories, and was consistently the

lowest for the severe category in seasons and varieties, variation within samples was also consistently the highest for the severe category in both seasons and varieties. Homogeneity is calculated by weighing the non-friable proportion of the flour from the friabilimeter, and the pattern of the results was the same as the friability: the intact, mild and skinned categories are highly homogeneous, with the severe category consistently scoring the lowest for both seasons and varieties. The highest number of unmodified whole grains was present in the severe category in both varieties and both seasons, with the severe category again having the highest proportion of the whole grains. In both harvest seasons Concerto had slightly higher friability, homogeneity and whole grains results than Chronicle, this difference between varieties was not statistically significant, as described in detail in sections 3.2.7 and 3.3.3.

Values of HWE were overall higher in the 2016 harvest season than in 2015 for both varieties. In both varieties and both seasons there is a clear pattern of increased extract with the increase in severity of skinning. In 2015 this pattern is observed up to skinned category, with the highest values for skinned and lowest for severe categories. In the 2016 harvest this pattern was for all four categories; HWE values were the highest for the severe category and the lowest for intact. However when the extract was adjusted for the biomass of the husk lost (AdjHWE), the pattern was reversed in both varieties and both harvest seasons and the extract obtained reduced with the increase in severity of skinning. In both harvest seasons and for both varieties the highest extract after adjusting for lost husk biomass was for the

intact category in both varieties, the lowest one was for the severe category in 2015 and the skinned category in the 2016 season.

The volume of the extract collected in 30 min of the filtration was much higher for all categories in 2016 harvest season, with the highest volumes collected for the intact and mild categories, with the exception of Chronicle in the 2016 harvest where the highest volume collected was for the skinned category; however both varieties had the lowest volumes in the severe category in both harvest seasons.

3.3.2.2 Malting losses

Malting losses were adjusted to a dry weight basis; results are presented in Table 3.3. Both years and varieties had the lowest malting losses for the severe category, as the root and acrospire growth in that category was limited. The highest malting losses in both years and varieties were recorded for the skinned category.

Table 3.3.Mean data from malt quality analysis of Concerto and Chronicle malt, harvest season 2015 and 2016

Year	Variety	Category	Skinning (%)	Friability (%) ± SD	Homogeneity	Whole grain	HWE ± SD	AdjHWE	Volume (ml)	M. Loss (%db)
2015	Concerto	Intact	<1.0	98.08 ± 0.48	100.0 ± 0.1	1.6 ± 2.1	303 ± 2.5	303 ± 2.5	182.8 ± 41.2	9.7 ± 1.0
		Mild	14.8 ± 3.6	99.96 ± 0.57	100.0 ± 0.1	2.0 ± 1.2	304 ± 4.3	301 ± 3.6	199.2 ± 39.7	9.9 ± 2.0
		Skinned	49.9 ± 4.6	99.68 ± 3.18	99.7 ± 0.6	7.3 ± 5.7	306 ± 5.3	281 ± 4.9	161.1 ± 49.8	10.6 ± 0.8
		Severe	84.5 ± 3.5	90.69 ± 17.86	98.1 ± 10.2	18.3 ± 6.3	289 ± 32.4	272 ± 31.1	100.6 ± 70.4	7.9 ± 2.8
	Chronicle	Intact	<1.0	97.6 ± 1.37	99.9 ± 0.2	2.8 ± 2.1	300 ± 4.2	299 ± 4.2	165.0 ± 39.1	12.7 ± 1.6
		Mild	18.9 ± 3.4	98.21 ± 0.32	99.9 ± 0.2	2.5 ± 2.4	305 ± 12.8	300 ± 10.8	159.6 ± 29.8	13.2 ± 0.4
		Skinned	51.8 ± 2.7	98.6 ± 0.42	99.9 ± 0.2	2.8 ± 1.8	308 ± 4.3	288 ± 4.1	160.6 ± 47.7	13.0 ± 1.4
		Severe	91.8 ± 3.2	86.1 ± 13.64	97.8 ± 8.0	43.4 ± 31.6	297 ± 16.1	279 ± 13.1	63.7 ± 19.1	9.2 ± 3.2
2016	Concerto	Intact	<1.0	97.62 ± 0.43	100.0 ± 0.0	0.0 ± 0.0	307 ± 4.2	306 ± 4.2	257.2 ± 24.5	8.0 ± 1.1
		Mild	18.5 ± 3.2	96.77 ± 0.56	99.7 ± 0.4	0.0 ± 0.0	307 ± 3.8	301 ± 3.8	239.4 ± 21.0	9.0 ± 0.5
		Skinned	49.3 ± 4.3	98.78 ± 0.46	100.0 ± 0.0	0.3 ± 0.6	313 ± 2.8	300 ± 2.7	235.0 ± 27.4	10.3 ± 1.1
		Severe	90.7 ± 4.0	96.96 ± 2.5	99.7 ± 0.2	3.0 ± 2.0	318 ± 3.1	303 ± 3.0	215.6 ± 43.8	8.4 ± 0.7
	Chronicle	Intact	<1.0	93.93 ± 1.09	99.5 ± 0.1	5.3 ±2.9	307 ± 5.1	306 ± 5.1	222.8 ± 22.8	9.5 ± 1.4
		Mild	18.0 ± 2.1	93.66 ± 1.08	99.4 ± 0.2	7.0 ± 2.7	305 ± 2.4	296 ± 2.3	206.1 ± 32.0	10.2 ± 1.5
		Skinned	51.0 ± 3.6	97.16 ± 0.45	99.7 ± 0.2	3.7 ± 1.5	312 ± 5.1	296 ± 4.8	231.1 ± 26.8	11.1 ± 0.8
		Severe	88.7 ± 1.9	90.67 ± 4.55	98.2 ± 0.9	18.7 ± 7.2	314 ± 5	301 ± 4.8	162.2 ± 22.8	9.1 ± 1.1

3.3.2.3 Skinning severity on HWE and adjusted HWE

The results of the mixed effect model used to determine the effect of variety and skinning category on HWE and AdjHWE are shown in the bar chart Figure 3.6. In addition, Figure 3.7 presents the model results with the replicates 2 and 3 of the 2015 micromalting year of severe category removed due to the obvious lack of modification in those samples, described above in section 3.3.2.

The interaction of variety and category was not statistically significant in testing for HWE and AdjHWE (P <0.05). The difference in HWE was significant only between the skinned and severe categories (*P* <0.001) and in AdjHWE in intact/mild and skinned/severe groups (Figure 3.6). When the replicates two and three were removed (Figure 3.7) the differences between the skinning categories in HWE and AdjHWE were not significant between the intact and mild (*P* >0.05) and skinned and severe categories (*P* <0.05), the significant difference was only between the intact/mild and skinned/severe category groups (*P* <0.05).

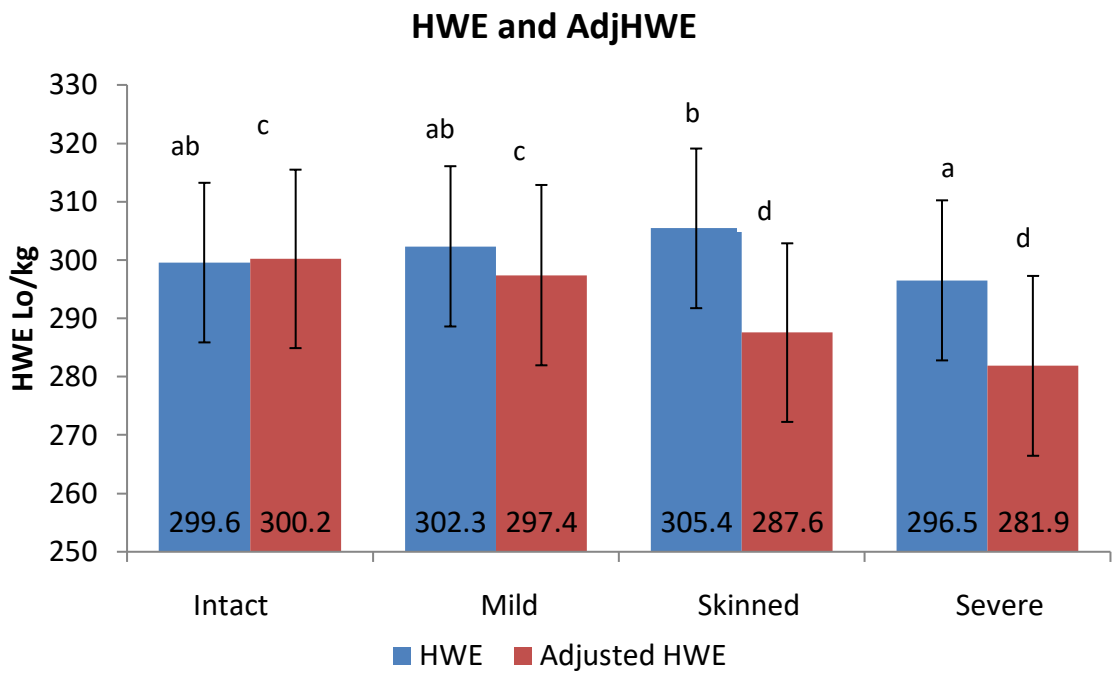


Figure 3.6. Model results of the effect of skinning category on the variables HWE and AdjHWE. The estimated means (values at the base of the bars) and 95% confidence intervals are plotted. Categories sharing a letter are not significantly different from eachother (pP <0.05)

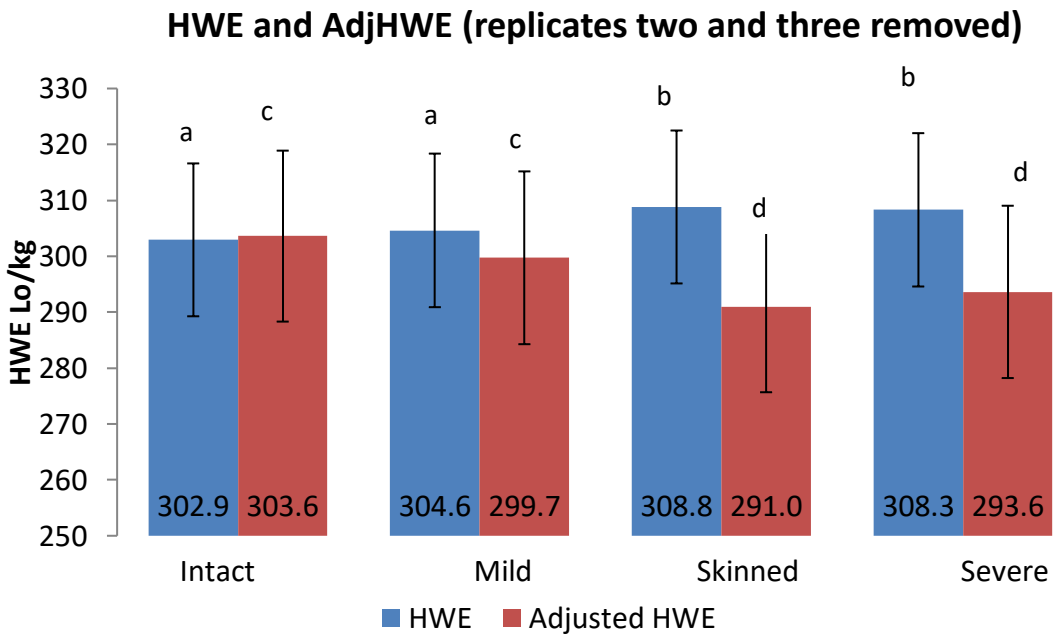


Figure 3.7. Model results of the effect of skinning category on the variables HWE and AdjHWE, after emoving the replicates two and three. The estimated means (values at the base of the bars) and 95% confidence intervals are plotted. Categories sharing a letter are not significantly different from eachother (P <0.05)

3.3.2.4 Skinning severity on friability and homogeneity

The interaction of variety and category was not statistically significant when testing for friability and homogeneity (P <0.05). The model results indicated that both friability and homogeneity had no significant differences among the intact, mild and skinned categories ($P > 0.05$); only the severe category was significantly lower ($P < 0.05$) than all the other categories (Figure 3.8. Model results of the effect of skinning category on the variables friability and homogeneity. The estimated means (values at the base of the bars) and 95% confidence intervals are plotted. Categories sharing a letter are not significantly different from eachother (P < 0.05)).

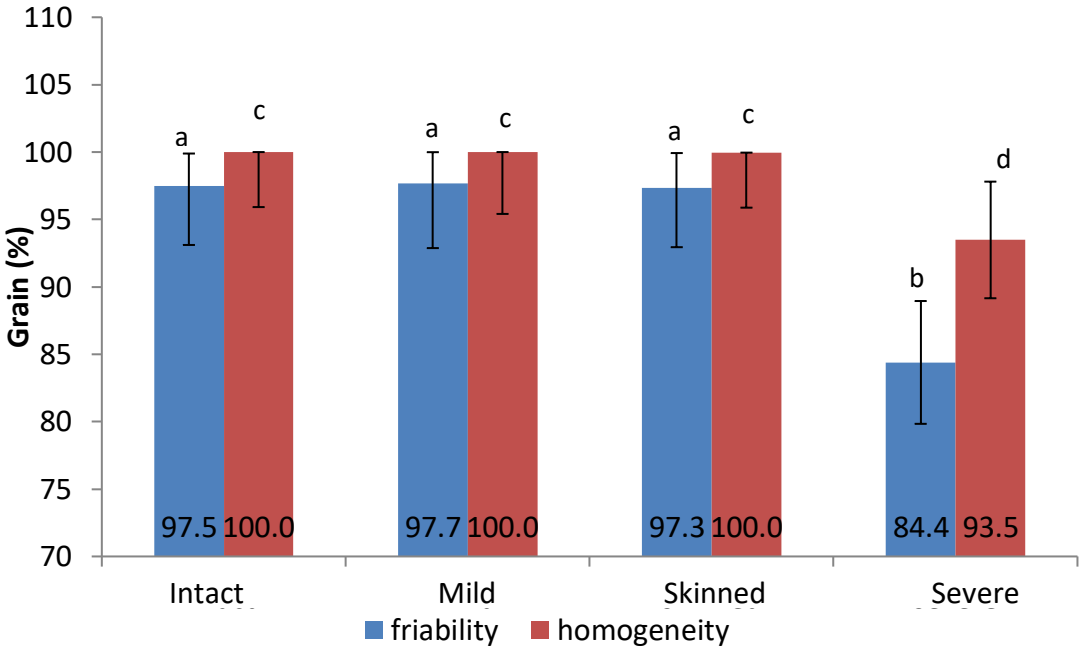


Figure 3.8. Model results of the effect of skinning category on the variables friability and homogeneity. The estimated means (values at the base of the bars) and 95% confidence intervals are plotted. Categories sharing a letter are not significantly different from eachother (P < 0.05)

3.3.3 Malt α-amylase

Overall in 2015 the α-amylase content was lower for all categories. In both harvest seasons the mild and intact categories had similar values, and the severe category had by far the lowest α- amylase content in both years, mean concentrations of α-amylase in 1 g of flour are presented in Table 3.4.

Table 3.4. Mean of three replicates of α-amylase concentration of the Chronicle malt

Year	Category	Concentration (Ceralpha Units/g Flour) ± SD
2015	Intact	164.32 ± 19.67
	Mild	169.76 ± 32.74
	Skinned	111.39 ± 20.61
	Severe	24.68 ± 19.17
2016	Intact	200.98 ± 9.84
	Mild	199.33 ± 17.06
	Skinned	194.64 ± 14.63
	Severe	133.91 ± 12.04

The interaction of variety and category was not statistically significant in testing for malt α-amylase concentration (P <0.05). Malt α-amylase content was similar for the intact and mild categories, the difference between them was not significant (P <0.05). The skinned and severe categories were significantly different from each other, and also from the intact and mild categories (P <0.05) (Figure 3.9).

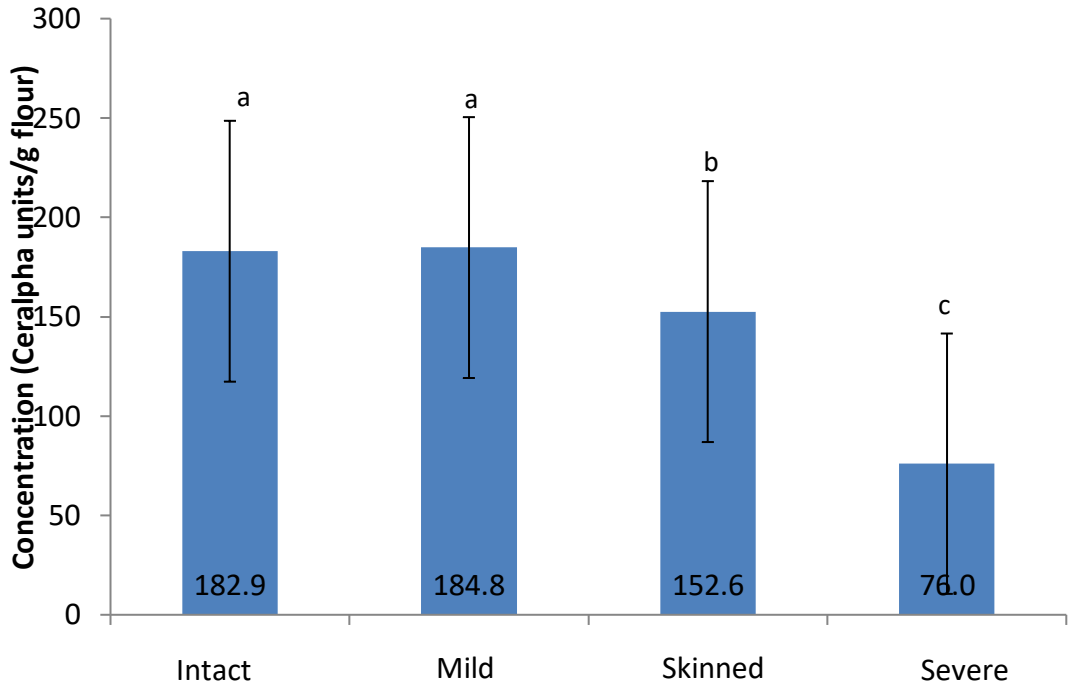


Figure 3.9. α-amylase content of Chronicle malt, model output data and CI's. variables sharing a letter are not significantly different from eachother (pP <0.05)

3.4 Discussion

Quality is the deciding factor in the approval of a batch of grains for malting, as only grains of the highest standard will be malted, those that do not meet the requirements have to be sold as feed barley, and growers must accept the loss of premium price. However some of the quality measures currently used can be influenced or even skewed by the presence of skinned grains. Grain analysis of Chronicle and Concerto with various skinning severities showed that TCW decreases with the increase in skinning severity, which is expected, as the husk constitutes on average 13% of the grain weight (Evers et al., 1999), and therefore if it is removed the grain weight will reduce. Even though it has been previously shown that grain size

does not always reliably indicate good quality of grains for malting (Bathgate, 1987), and more recently by Yu et al., (2017), who suggests that starch structure is a more reliable predictor of good malting barley. Nevertheless, maltsters prefer large and plump grains and high SW, which is usually interpreted as such, and does positively correlate with grain plumpness (Edney et al., 2005). In the current study, categories with high levels of skinning increased in SW, but decreased TCW, which signifies that the grains are smaller. Increased SW suggests that small grains have a different packing properties and pack more closely together; therefore interpreting SW should always be done with consideration to the levels of skinning and TCW of the sample. It has been previously shown by Hoyle et al., (2018) that SW is a combination of different factors, including grain size, density and packing efficiency and the interaction of this factors is crucial.

Germination tests are used to examine the viability of the grains (H_2O_2 test); dormancy and homogeneity of germination, to ensure all the grains germinate at similar rate (4 ml test), and lastly to check for water sensitivity (8 ml test). Water sensitive barleys have a higher requirement for oxygen, which has previously been shown to be at least partly due to the presence of large numbers of microbial species (Gaber & Roberts, 1969), and such grains are not able to germinate if the surface is covered with a film of water (Crabb et al., 1968), resulting in lack of germination . As previously discussed the presence of the husk is necessary for the protection of the embryo, and in these experiments a slight decrease of viability of the embryo was observed for the skinned and severe categories, which was most likely attributed to the damaged embryo. Industry expects the germination to be above 98% and the

levels of germination in severe category were within those expected levels (MAGB, 2019). Germination results for 2016 harvest season were overall better than those for the previous year, which was also translated into better micromalting performance of the 2016 samples, and this will be discussed in more detail later in this section.

Micromalting took place in different units; in 2015 it was conducted at Heriot-Watt University (HW) and in 2016 at the Scotch Whisky Research Institute (SWRI), due to technical problems with the unit at HW (these occurred long after my own malting experiment and are therefore not expected to affect my results in any way). Modification of the samples during the germination stage of micromalting, before the samples are kilned, can be visually assessed by inspecting the vigour of rootlet and acrospire growth (Agu et al., 2012). In the 2015 harvest sample from the severe skinning category did not modify in replicates two and three. These samples were overgrown by bacterial and fungal infection, and this is similar to the findings of Meredith (1958) who also observed excessive microbial growth in categories with large proportions of skinned grains. The exposed endosperm provides an easy breeding ground and access to nutrition for the microflora and resulting in the increase in their numbers. Barley grains, which have been affected by other disease e.g. mildew, are likely to skin more and this has been observed during this project, but have not been quantitatively or qualitatively assessed, and there is no previous research on the influence of other diseases on skinning. However, mildew often occurs on grains with increased moisture, and moisture is a factor that exacerbates skinning in otherwise healthy grains, making it difficult to point which of the factors

is responsible for exacerbated skinning. The growth of rootlets for the severe category was minimal, unlike the other categories, and this has been previously attributed to presence of excessive microflora (Kelly & Briggs, 1992b). Although the microbial colonies in barley and malt in this study were not investigated, the increase in growth was very obvious and visible on the grains and the accompanying smell was also clearly microbial. Interaction of microflora, barley and malting process is very complex and bacteria and fungi influence it in many ways, including competition with the germinating grains for the nutrition and oxygen, and production of enzymes, hormones and toxins that affect the germination and malting process, but also influence the quality of final malt and interfere with the brewing and distilling process, contributing to reduced extract values and poor filtration, and reduce the levels of α-amylase in final malt (Noots et al., 1999; Rautenbach, 2014; Kelly & Briggs, 1992b; Gaber & Roberts, 1969; Doran & Briggs, 1993; Briggs & McGuinness, 1993). Replicate one in 2015 and all three replicates of 2016 have modified well; this difference is likely to be due to the different malting units used, the one at SWRI is a more modern one, with automated grain mixing and as opposed to the HW, where mixing is done manually, increasing the risk of introducing infections. Microbial growth might have been partly responsible for the reduced germination, but the difference was also likely due to the natural variance between harvest years. The germination tests suggest that the severe category in 2015 had poorer germination results than 2016, even though the preparation of the categories was the same in both years.

In the micromalting experiments skinned and severely skinned categories had increased damage to the embryo, impairing the germination. Although the specific damage to the embryo was not examined during micromalting studies it was suggested by MacLeod &Palmer (1966, 1967) that damage to the nodal region in the embryo, located between the root and acrospire could be responsible for the different germination patterns observed in skinned and severely skinned grains in micromalting experiments. This highlights the protective role of the husk in germination of the barley grains. Damage to the various regions of the embryo results in altered patterns of germination and root and acrospire growth, in addition affecting the modification (MacLeod & Palmer 1967). It was highlighted in Chapter 2 that there are two different types of husk loss, dependent on the moisture content of the grains. It is possible that this also plays a role in the severity of embryo damage. Force required for the grains to skin could differ depending on the type of husk loss occurring, and this needs to be further investigated. Higher force required for the husk to be removed may increase the likelihood of embryo damage or damage to other organs.

In this study the malting program was not adjusted for the categories with a large proportion of skinned grains, some studies suggest that it is possible to obtain malt of great quality by adjusting the steeping program to use less water and prevent the grains without husks from drowning (Agu et al., 2009; Swanston & Middlefell-Williams, 2012). However in this project it was important to compare those extreme circumstances with other categories during a normal, non-adapted malting cycle, and even though skinning created in the severe category would be unlikely to occur

naturally in the field, this study was the first to investigate the effect of skinning on malting in modern barley varieties, therefore the aim was to find if skinning does make any difference at all, and therefore using less extreme categories, could have averaged out the small differences.

Hot water extract is the main indicator of malt quality used to assess malted barley in the UK, and it has been shown to be positively correlated with malt quality (Gianinetti et al., 2005; Fox et al., 2003; Molina-Cano et al., 2002). High HWE of the malt is often interpreted as the barley sample having all the desirable qualities: large, plump grains, low in protein and rich in easily digestible starch, germinating homogeneously with high enzymatic activity able to rapidly breakdown the starch into simple sugars (Briggs, 1998). The results from the experiments conducted in this project show an increase in the HWE in the categories with high proportions of skinned grains, which if considered just on its own, would suggest the above statement to be true. However, when we carefully examine all other measures of malt quality, including friability, homogeneity, the volume of extract collected, and the α-amylase content, they all suggest a very poor quality of malt. Standard measures of malt quality have previously been shown as inadequate by Palmer (2000), who concluded that the homogeneity of modification is correlated with the protein content of the grains. In addition, as evident in current study, high proportion of skinning in the bulk can obscure the true value of HWE. Only after accounting for skinning this obscuration becomes visible. Similarly, it is possible to obtain the same value of HWE for malts that are modified to differently or have a damaged embryo.

The important question that needs to ask at this stage is why the HWE is inflated in the severely skinned categories, if it is clearly malt of inferior quality? The answer to this question is fairly straightforward and lies in the methods used for HWE measurement, which use a standard weight (50 g) of the sample. This over inflation of HWE is due to an increased proportion of starchy endosperm in the skinned samples, previously observed also by Swanston et al., (2011). Husks constitute on average 13% of the grain weight (Evers et al., 1999), but have no sugary value to add to the HWE, therefore 'diluting' the sample. In this study, the over inflation of HWE is obvious because such extreme categories were used. Barley varieties currently approved for malting only vary from each other by 1-2 l^o/kg (AHDB, 2019), and if this was a malting trial for barley variety selection, a difference of 1 l^o/kg in HWE can often be the deciding factor in new variety being accepted or rejected, and this difference could easily have come from one sample skinning more than the rest. To address and better represent the changes in extract, this study used a measure first suggested by Meredith (1958), where HWE was adjusted according to the number of grains in the sample rather than the weight, however the exact calculation used in their study was not published. With this in mind, to present the HWE per number of grains instead of per 50 g sample, the difference in TCW between the skinned categories and the intact sample was used to represent different levels of husk-loss compared to the perfect scenario, with all the husks in place. When the HWE calculation was adjusted for husk-loss, it was evident that the pattern of AdjHWE was the reverse of HWE, with extract decreasing as skinning severity increased. This observation suggests that a large number of grains have not modified, or possibly over modified resulting in the

extract from those grains being unrecoverable. It would be beneficial for the malting industry if future studies focused on developing a factor which could be applied to skinned samples, allowing for adjustment of the husk biomass lost. Further discussion on modification of the grains in presented in the friability and homogeneity section below.

Volume of the extract collected is an additional measure taken during preparation of the samples for the HWE, and uses the volume of extract collected in 30 min. Worts produced from poor quality malts are high in β-glucans, which make the wort viscous and slow down the filtration (Scott, 1972; Swanston et al., 2011). The volume of the extract collected can vary depending on the type of filter used and it is not reliable measure for comparing between the varieties or even between different harvest seasons. In this project it was useful to look at the differences between the skinning categories within the same variety and the same harvest season. In this case, it is clear that the extracts from the severe category have much slower filtration rate compared with intact, mild and skinned grains, which adds evidence to all other measures used, about the inferior quality of the severe malt in this study.

Hydrolytic enzymes in barley grains break down the starch to simple sugars, which are easily digested by yeast later in the process of brewing and distilling (Sammartino, 2015; Briggs, 1998; Fox et al., 2003). There is a large number of enzymes acting to hydrolyse the starch, the most abundant are: α-amylase, which is synthesised in the germinating grains *de novo* and which cleaves the long chains of

amylopectin in random locations, shortening the chains of oligosaccharides; and β-amylase, which is present in the endosperm of barley grains in its inactive form, when it is activated during germination it acts on the second α-1,4 bond from the non-reducing end producing the disaccharide maltose (Sammartino, 2015). The absence of α-amylase from the grain until the germination takes place, made it a better enzyme to study as a predictor of how well the grains modify, unlike β-amylase, which levels would not be expected to be affected by skinning.

The levels of α-amylase in malt produced from the skinned grain reduce with increase in skinning severity, which supports the other evidence in this study, suggesting that severely skinned grains did not germinate and did not modify as well as intact grains. In addition, the levels were much lower for all categories in 2015 harvest season and this is possibly due to one of the two factors, or combination of them. Variation between harvest seasons and therefore growing conditions could plays a part and growing environment has previously been shown to strongly correlate with the levels of α-amylase (Arends et al., 1995; Fox et al., 2003). In addition, this difference could potentially be due to poor stability of α-amylase in the stored malt. The measurement of α-amylase levels took place later in the project, by that time the 2015 harvest samples have been over one year old, and the 2016 samples were only a few months old, and although there are evidence that storage of barley prior to malting does not affect enzymes (Reuss et al., 2006), the research on the effects of storage on malted grains is lacking and should be the focus of future projects. The samples which showed excessive microbial growth in 2015 season, showed the lowest levels of α-amylase which agrees with the findings of Kelly &

Briggs, (1992), that excessive microbial growth inhibits the synthesis of α-amylase in germinating grain.

Friability is a good predictor of malt quality and of modification in the bulk of grains (Bathgate, 1983; Wentz et al., 2004; Edney & Langrell, 2004) The results show a good friability levels for the intact, mild and skinned categories, and this levels would be acceptable for industry malt samples; grains from the severely skinned category however are below acceptable score for friability. Even though the results show around 91% friable grains, this measure tends to overestimate friability and homogeneity (Briggs, 1998; Darlington & Palmer, 1996). The results suggest that homogeneity was good for all the samples. This method is a quick industry standard for large bulks, and gives an overview of the modification of the bulk, but it does not provide any detail on single grains. This was further investigated using Carlsberg Malt Modification method and is described in Chapter 4.

3.5 Conclusions

The quality of barley grains influences the quality of malt produced and the efficiency of the whole malting process. This study was the first one to quantify the detrimental effects of skinning on the quality of malt produced. It is important to ensure that no single measure of malt quality is interpreted on their own, especially as this was shown in the current study with HWE, which was inflated in skinned categories but when this was adjusted to account for the husk-loss and interpreted with other

measures of malt quality, the malt from skinned grains was of the inferior quality. In future it would be beneficial to develop a factor which could be applied to skinned barley bulks, allowing the industry to better evaluate the quality of grains and adjust for the missing husks.

Chapter 4. Effects of skinning on modification

4.1 Introduction

Previous chapters have demonstrated that skinning results in inefficiency in malting and affects the quality of malt produced. This chapter considers in more detail how different levels of skinning affect modification during the malting process. Measurements on single grains will determine if there is any evidence of under- or over-modification, which would explain the production of poor quality malt.

The main objective of malting is to produce and release of enzymes responsible for breaking down the cell walls surrounding the starch granules which are stored within the endosperm cells, in a process known as endosperm modification. Ideally this process completes with minimal starch degradation. A well modified endosperm is necessary, as later during mashing in the brewery or distillery the starch gets hydrolysed into mono- and polysaccharides mainly by α- and β-amylases. Yeasts then convert these simple sugars to ethanol during fermentation.

Enzymes responsible for breakdown of cell walls in the endosperm are either activated or produced during germination, as described in detail in chapter 1; this chapter considers modification as influence by skinning. It is important to mention that the release or production of the enzymes usually starts at the scutellum and

progresses through the aleurone layer from the proximal to distal end of the grain, there are however two theories of how this happens advocated by two scholars. Briggs suggested that modification progresses parallel to the aleurone layer (Briggs, 1972; Briggs & Macdonald, 1983) and Palmer proposed that modification progresses from the junction of aleurone and scutellum (Macleod & Palmer, 1968; Palmer, 1982). Schemes of both theories are represented in Figure 4.1. The scientific community is still divided on how modification progresses, and it is possible that both theories are correct, depending on the variety or even an individual grain within the batch, both models might be applicable (O'Brien & Fowkes, 2005).

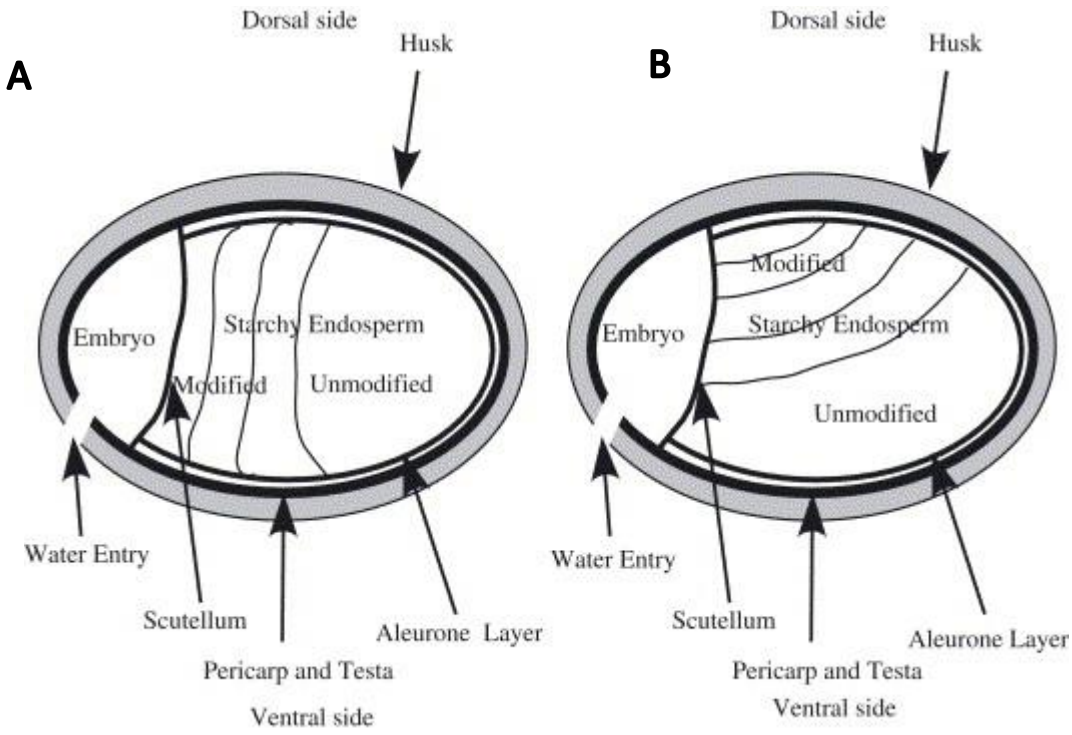


Figure 4.1. Representation of two theories of progression of malt modification as proposed by Briggs (A) and by Palmer (B). Source (O'Brien & Fowkes, 2005)

The first hypothesis explaining the inefficiency in malting considers undermodification to be the main the source of lack of efficiency. Friability and homogeneity results reported in this thesis, and discussed in chapter 3, suggest this to be true. However these two tests use a large sample of grains and are not specific enough to look at a single grain level modification or to distinguish unmodified from case-hardened grains (Thomas, 1986). Another theory suggests that skinned grains take up water faster and could modify and germinate faster, resulting in overmodification of grains and lack of homogeneity within the sample (Bryce et al., 2010); it was also speculated that barley husk reduces the availability of oxygen to the embryo, and therefore lack of husk would result in faster germination (Lenoir et al., 1986). It was necessary to look in detail at a single grains for signs of overmodification and undermodification and definitively indicate whether one mechanism or both is responsible for the poor performance of skinned grains in malting.

Undermodification of grains is the result of lack of germination or incomplete modification of grains. As discussed in chapter 1, an intact embryo is necessary for germination to take place and for the grain to fully modify. Skinned grains are at higher risk of having damaged embryos due to the lack of husk. In addition during germination in malting the acrospire grows under the husk and is further protected through these first days of development (Figure 4.2).

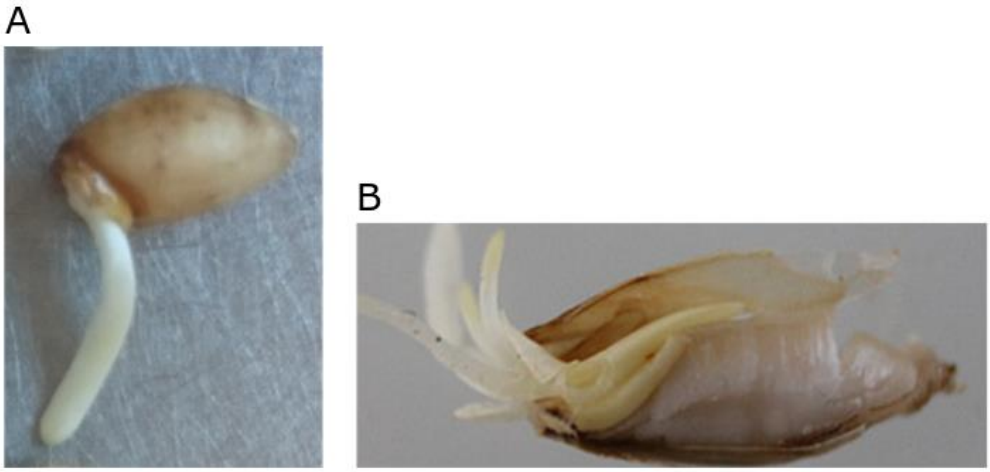


Figure 4.2. Difference in acrospire growth between skinned grains (A) and grains with intact husk (B)

In the skinned grains the acrospire grows outwards and is easily knocked off during routine mechanical agitation of the malt, and when this happens the modification halts. Methods used to assess the undermodification of the grains include friability and homogeneity, which were described in detail in chapter 3, and are a very useful tool in estimating modification of the large bulks of the malt. Detailed studies into the modification of single grains can be done using the Carlsberg method developed by Aastrup (Aastrup, 1988; Aastrup et al., 1981), in which calcofluor is used to bind to unmodified β-glucan in the endosperm cell walls, and this fluoresces under the UV light. Another method to investigate single grain modification was developed by Palmer (Palmer, 1975a; Roberta & Palmer, 2005), in which malted grains are cut in half longitudinally and mashed at 65^{o}C, allowing modified endosperm to disperse, leaving unmodified regions of the grains intact. In this project the Carlsberg method was used, due to ease of access to the equipment and better accuracy of the calcofluor measurement.

Overmodification occurs when the grains are allowed to germinate for too long and the starchy endosperm of growing grains begins to break down. The developing seedling uses the starch stored in the endosperm to support the growth of the acrospire and the roots. This leaves less starch available for hydrolysis into monosaccharides during mashing and for yeast to convert into ethanol during fermentation. Overmodified malt is problematic during transport – it breaks easily and produces large quantities of dust, which is hazardous to people working with malt, and extraction of dust adds costs. Beer produced from overmodified malt has poor head retention, and has thin character, lacking body (Briggs, 1998). It is important to halt the modification by kilning the grains at the right moment. The visual assessment method used by maltsters to assess modification in grain with intact husk, assumes that when the acrospire is about ¾ of the grain length, the grain should be fully modified (Briggs, 1998). If the acrospire is allowed to protrude at the distal end of the grain, from under the husk, overmodification has occurred. Acrospires in skinned or naked grains grow in a different manner, as described in the above section.

Unlike undermodification there are no standard methods to measure overmodification of the grains. Maltsters use the above visual assessment of the barley acrospires (Briggs, 1998), however this is not quantitative. Malting losses is also used to assess overmodification, as overmodified grains have higher root/ acrospire biomass, therefore resulting in lower weights of starchy endosperm. Methods developed in this project for assessing overmodification were based on the changes in malting barley grains and its physiology previously described by other

researchers (Kano et al., 1981; Bathgate & Palmer, 1973; Palmer, 1972a). Physiological changes in the malting barley grain start with enzymatic breakdown of the cell walls surrounding starch granules in the barley endosperm followed by the hydrolysis of the small starch B-granules. If modification is allowed to progress for long enough, pitting of the large starch A-granules would occur, as the grain germinates and hydrolyses its energy stores. These three elements of endosperm modification were observed and scored after examining micrographs taken by scanning electron microscopy (SEM). Overmodification of the grains results in the breakdown of the starch into simple sugars, which are the source of energy for the development of the plant. During mashing starch is converted into maltose and glucose; maltose being the main sugar for yeast to convert to ethanol. Excess glucose would be the result of overmodified grains and it has been linked to poor performance of the yeast in brewery and distillery. Measurement of monosaccharides in the malt extract was another method I used to investigate overmodification of the malted grains, and thin layer chromatography was the available method for quantitative measurements of the monosaccharides (MacLeod et al., 1953; Evans et al., 2005; Mcgorum et al., 2012).

4.1.1 Aims and objectives

The aim of this chapter was to investigate modification of grains in malt samples obtained from the grains with various severities of skinning. The objectives were to i) examine undermodification in single using calcofluor method; ii). to quantify under-

and over-modification in malt extracts by measuring mono- and oligo-saccharide composition using thin layer chromatography (TLC) and iii). to further assess overmodification by scoring malt structural changes using scanning electron microscopy.

4.2 Materials and methods

4.2.1 Calcofluor (Carlsberg) Malt Modification - undermodification

Sample Preparation

Samples of Concerto and Chronicle malt from experiments described in chapter 3 were used. The method used was based on original Carlsberg malt modification method (Aastrup, 1988; Aastrup et al., 1981), which uses calcofluor to bind to β-glucan in unmodified parts of the endosperm. The Malt Modification Analyser is located at James Hutton Institute, Invergowrie, Dundee (IMAGE HOUSE A/S, Copenhagen) (Figure 4.3).

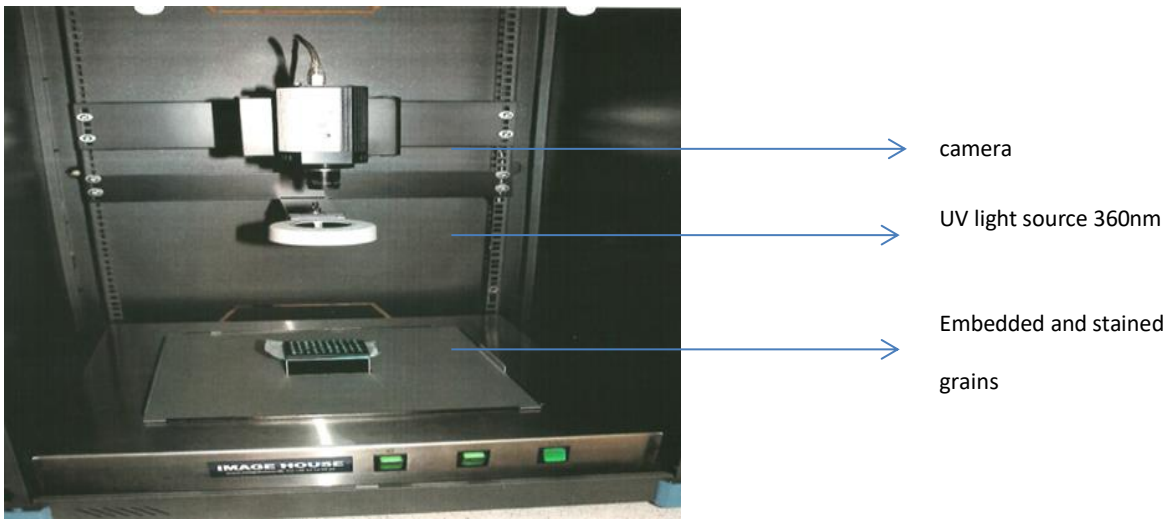


Figure 4.3. Malt Modification Analyser at James Hutton Institute, Invergowrie, Dundee (IMAGE HOUSE A/S, Copenhagen).

A sample of 50 grains from each variety and each category was shaken into a slot on the seed tray (Figure 4.4A) and then pressed into a block of black polymer clay (Cernit Number One 56g, Cernit UK) (Figure 4.4B) and embedded approximately halfway by pressing the grains into the clay with a purpose-built press (Figure 4.4C). A belt sander was used to sand down the top half of the grains (Figure 4.4D). The plates were then stained with Calcofluor white (Fluorescent brightener 28, Sigma Aldrich F3543-1g) for 30 s, rinsed with 70% ethanol and counterstained with Fast Green FCF (Sigma Aldrich F7252-5g) and blot dried. The plates were allowed to fully dry overnight before being placed in the malt analyser.

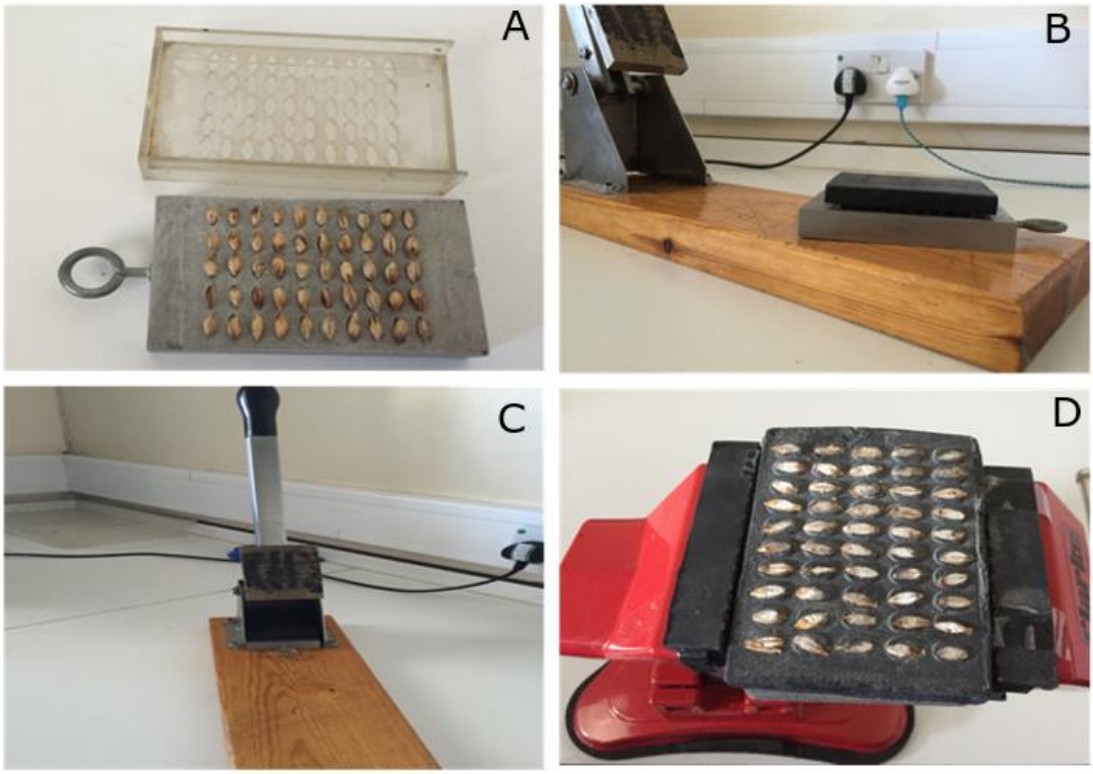


Figure 4.4. Embedding process. A -Seed tray with 50 grains shaken into the slots; B – black clay block placed on top of the seed tray; C – purpose build press for embedding the grains into the clay; D – embedded grains sanded down approximately ½ way.

Malt Modification Analyser Output

The contrast for white and black was manually adjusted. Analysis produced an output of three spreadsheets and an image of the plate; sample spreadsheets is described below and Figure 4.5 is a sample image output.

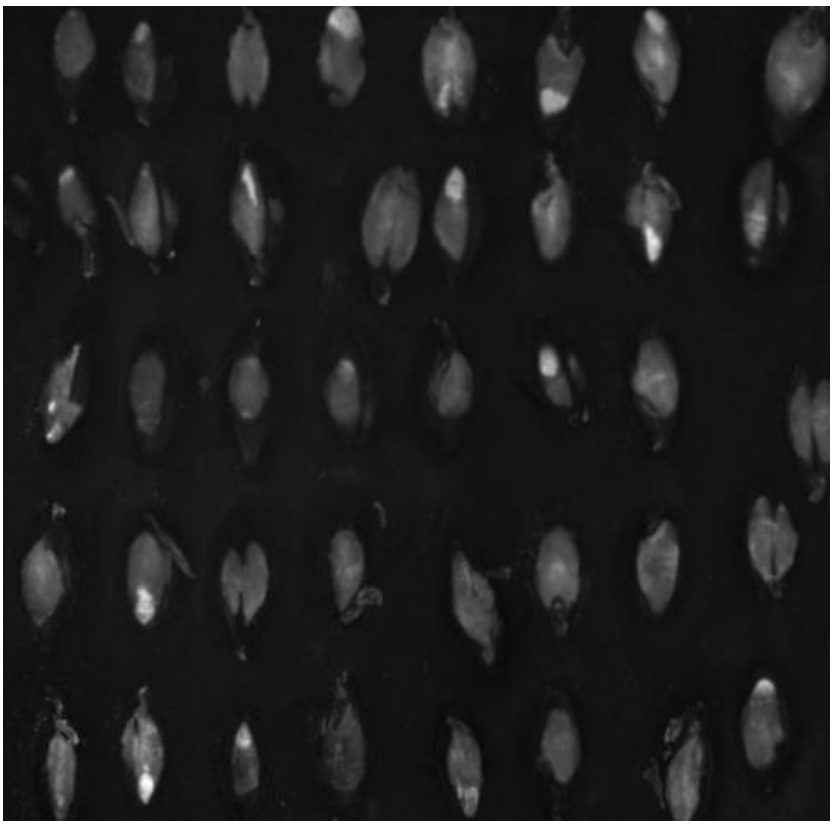


Figure 4.5. Sample image output from Malt Modification Analyser. Malted barley grains with their endosperms exposed by application of a sanding belt were stained with calcofluor and imaged under UV light; white regions of the grains are not modified.

The spreadsheets were: Malt White Percentage, Malt White Class and Malt White Score. Malt White Percentage is the result of a calculation of the unmodified area of the grain, in proportion to the size of the grain; Malt White Class, was an output where each grain was assigned into a class ranging from Class 0, with high proportion of undermodification to Class 5, in which the grain has fully modified; Malt White Score showed three values: M, H and A; M stands for modification, H stands for homogeneity and A is a coefficient number calculated in the original Carlsberg method (Aastrup et al., 1981; Aastrup, 1988). The values for M are calculated based on the classification of the grain into one of the categories of modification in Malt

White Class sheet. Calculation of H is based on M and A; in the original manual for the malt analyser the formulas used for those calculations were not shown, and were only supplied after a contact with the author of the manual. Malt White Percentage was the output used in this project, as it was a quantification of the proportion of unmodified regions of individual grains, which allowed for statistical analysis of the results.

4.2.2 Thin layer chromatography – overmodification and undermodification

This method is based on the method previously described by Mcgorum et al., (2012). Hot water extracts (HWE) from the micromalting studies were separated using thin layer chromatography with Merck silica gel '60' plates (Merck KgaA, Darmstadt, Germany). For each sample the HWE was diluted 1:2 and 1:16 with distilled water. Two dilutions were needed as some sugars (xylose and arabinose) were not visible at high concentrations; whereas maltose was too concentrated at low dilutions and the spots were only of appropriate quality at 1:16 concentration. Sugars used as a control were: rhamnose, xylose, arabinose, fructose, glucose, galactose, sucrose, maltose and maltotriose. Sugar standard was prepared at a concentration of 15 µg/l of each sugar, and this was then serially diluted by 3.5 (v/v) with distilled water giving concentrations of 4.29 µg/l, 1.225 µg/l, 0.35 µg/l and 0.1 µg/l. Distilled water was used as a negative control. Each plate was loaded with 3 µl of diluted extract and with standard curve of monosaccharides. The plates were developed twice in ethyl

acetate: pyridine: acetic acid: water (6:3:1:1 by volume) (EPAW). Stain used for visualisation of the spots was thymol (0.5% (w/v)) and concentrated H_2SO_4 (5% (v/v)) in ethanol. Plates were briefly dipped in the stain, dried and placed in the oven at 105°C for approximately 5 minutes. Samples were run in triplicate.

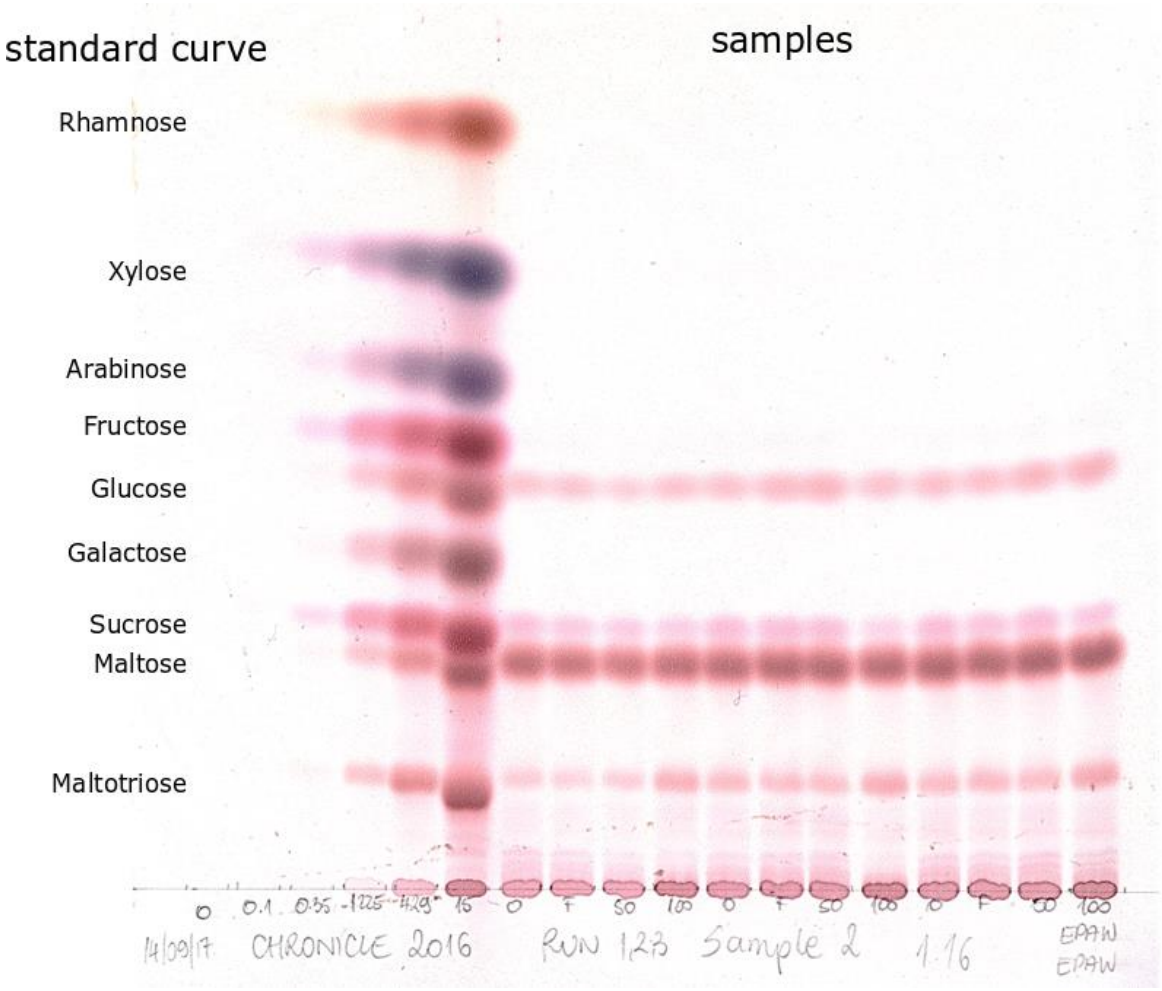


Figure 4.6. Sample TLC plate with sugar standards on the left and samples diluted 1:16 on the right.

Scans of the developed plates were analysed using Photoshop CC (Adobe, San Jose, California, United States), previously described by Mcgorum et al., (2012). The

ellipse tool was used to measure mean pixel intensity of the spots on the standard curve and in the unknown samples using 'histogram' tool on the 'green' channel. The values obtained were subtracted from the background mean pixel intensity and obtained values were input into GraphPad Prism 7 (GraphPad Software, La Jolla, CA). This software allowed for interpolation of the unknown values from the standard curve, the best fitting line was a hyperbola.

4.2.3 Scanning electron microscopy – overmodification

Samples of Chronicle malt from years 2015 and 2016 malting experiments described in chapter 3 were used. Ten grains from each of the micromalting runs were analysed using scanning electron microscopy (SEM) to visually score the signs of overmodification. Grains were cut in half longitudinally and mounted on a stub using an adhesive carbon disk. The stubs were then coated with gold. Each grain was evaluated in three distinct sections: proximal, middle and distal ends. Three distinct elements of modification were scored: cell wall degradation, A-granule pitting and B-granule degradation were scored on a scale of one (none observed) to five (severe degradation/pitting), from three sections of the endosperm in each grain. Overmalted category was prepared for this experiment by placing 10 intact grains from each of the malting years on the 9 cm diameter Petri dish lined with two filter papers, followed by addition of 4 ml of tap water. The plates were incubated for 5 days at 18^{o}C, until the acrospire has grown well beyond the length of the grain. The grains were then air dried and prepared for SEM in the same way as malted grains.

Unmalted grains were used as negative control and visibly overmalted grains were used as positive control.

4.2.4 Statistical analysis

Statistical analyses were conducted using R (R Core Team, 2016) programming language. Linear mixed effects models were fitted using package 'lme4' (Bates et al., 2015). In the undermodification experiment the effects of the dependent variables 'variety' and 'category' and their interaction as the fixed effects, and 'year', 'sample' and 'run' as random effects on the undermodified area of the grains were tested. In thin layer chromatography experiment, dependent variable fixed effects were 'sugars', 'variety' and 'category' and 'year', 'sample' and 'run' as random effects. Finally in scanning electron microscopy linear mixed effect model with family 'Poisson' from package 'lme4' was used (Bates et al., 2015). Fixed effects were 'category' and 'position within the grain' and their effect on overmodification score. Random effects were 'grain number', 'position' and 'year'.

The minimally adequate models were chosen by comparing hierarchical models using ANOVA ($\alpha = 0.05$) and significant differences among the effects were determined by least squares means pairwise comparisons using package 'emmeans' (Lenth, 2019).

4.3 Results

4.3.1 Undermodification – Carlsberg method

An increase in severity of skinning resulted in the increase of mean unmodified area of the grain for both varieties. The highest proportion of unmodified grains was in the severe category and the lowest in the intact category; the mean results are presented in Table 4.1. The variation within the samples (as expressed by standard deviation) also increases with the increase in severity of skinning. This pattern is clearest for the variety Concerto, where the lowest variation is in the intact sample and the highest in the severe samples. In the variety Chronicle the lowest variation is within the mild sample, and the highest in the severe category.

Table 4.1. Mean ± SD of unmodified area of the grain for each variety and skinning category

Variety	Category	Mean unmodified area (%) ± SD
Chronicle	intact	3.94 ± 12.71
	mild	6.53 ± 12.09
	skinned	13.25 ± 15.77
	severe	19.15 ± 18.84
Concerto	intact	1.54 ± 5.27
	mild	4.67 ± 9.35
	skinned	13.26 ± 15.69
	severe	19.87 ± 21.48

Variety and category were statistically significant variables (P <0.001), and their interaction was not significant (P >0.05), details are presented in Figure 4.7.

Intact category was not significantly different to mild category (P >0.05) but it was better modified than skinned or severe (P <0.05). Mild category was also significantly better modified than skinned and severe categories (P >0.05) within the variety. Skinned and severe categories were not significantly different to each other for both varieties (P >0.05).

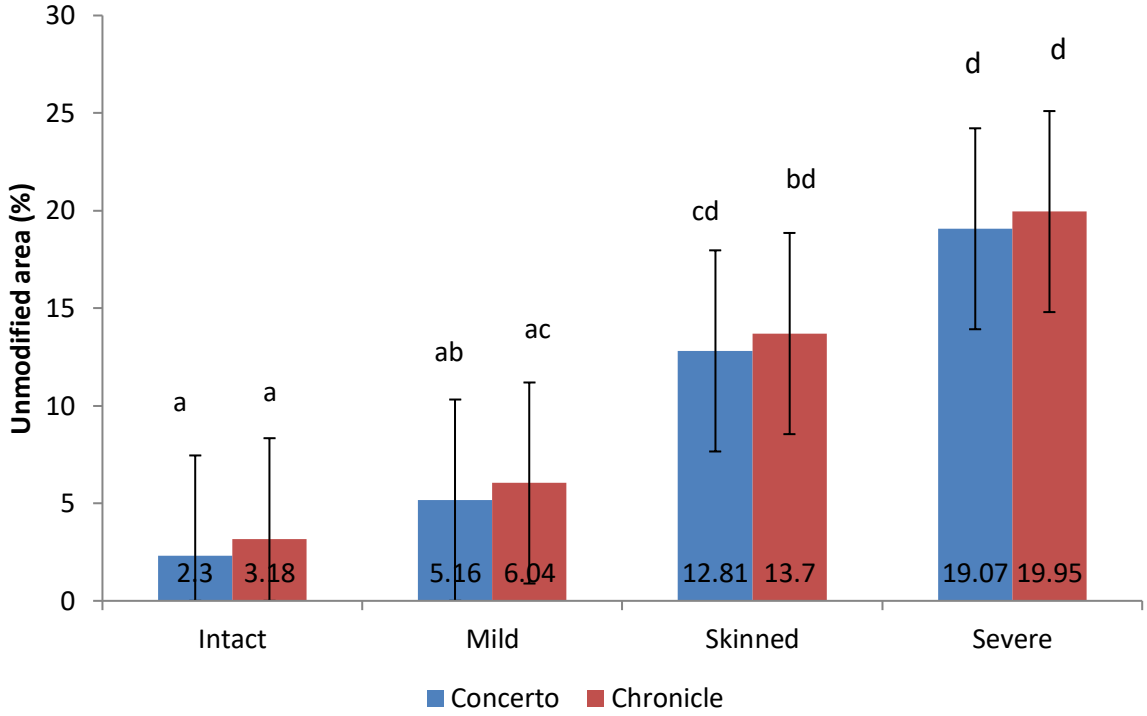


Figure 4.7. Malt modification analysis estimated values. White (unmodified) area of the grains for each skinning category, categories sharing a letter are not significantly different from each other (P >0.05), 95% confidence intervals have been plotted.

4.3.2 Overmodification and undermodification – thin layer chromatography

Analysis of the composition of the hot water extracts showed various quantities of monosaccharides. Rhamnose and galactose were not detected at all. The lowest concentrations were for arabinose and xylose, and highest for maltose, in both varieties and across the skinning categories, mean values and standard deviation of the monosaccharide concentrations are presented in Table 4.2.

Table 4.2. Mean concentration (±SD) of sugars for Concerto and Chronicle in intact, mild, skinned and severe skinning categories

		Chronicle	Concerto
Skinning	**Sugar**	**Mean conc. (g/L) ± SD**	**Mean conc. (g/L) ± SD**
Intact	xylose	0.07 ± 0.02	0.05 ± 0.01
	arabinose	0.08 ± 0.03	0.06 ± 0.02
	fructose	0.22 ± 0.04	0.21 ± 0.03
	glucose	2.51 ± 0.41	2.80 ± 0.47
	sucrose	1.47 ± 0.13	1.59 ± 0.22
	maltose	15.17 ± 1.90	15.74 ± 3.37
	maltotriose	2.51 ± 1.03	2.53 ± 0.81
Mild	xylose	0.07 ± 0.02	0.05 ± 0.02
	arabinose	0.07 ± 0.02	0.06 ± 0.02
	fructose	0.21 ± 0.03	0.19 ± 0.03
	sucrose	1.38 ± 0.20	1.43 ± 0.18
	glucose	2.43 ± 0.29	2.64 ± 0.49
	maltose	15.44 ± 2.19	15.29 ± 2.77
	maltotriose	2.72 ± 0.97	2.73 ± 0.83
Skinned	xylose	0.05 ± 0.02	0.05 ± 0.02
	arabinose	0.05 ± 0.02	0.06 ± 0.02
	fructose	0.18 ± 0.03	0.18 ± 0.05
	glucose	2.28 ± 0.62	2.80 ± 0.67
	sucrose	1.29 ± 0.13	1.43 ± 0.22
	maltose	15.04 ± 2.82	15.05 ± 3.35
	maltotriose	2.78 ± 0.89	2.78 ± 0.95
Severe	xylose	0.04 ± 0.02	0.04 ± 0.02
	arabinose	0.04 ± 0.03	0.05 ± 0.02
	fructose	0.13 ± 0.05	0.18 ± 0.03
	glucose	2.04 ± 0.62	2.88 ± 0.53
	sucrose	0.88 ± 0.19	1.19 ± 0.20
	maltose	14.99 ± 2.17	15.11 ± 3.08
	maltotriose	3.43 ± 0.69	3.58 ± 0.80

The interaction of variety and skinning category was significant for xylose, arabinose, fructose, glucose and sucrose (P <0.05); for maltotriose only the skinning category was significant (P <0.001) and for maltose there was no significant

difference between any of the skinning categories and any of the varieties (P >0.05), details are presented in Figure 4.8. Concentrations of mono- and polysaccharides in malt hot water extract for Chronicle and Concerto varieties, with varying skinning levels. Categories and varieties sharing a letter are not significantly different from each other (P >0.05), 95% confidence intervals have been plotted. Xylose and arabinose are monosaccharides associated with cell wall breakdown, and their concentration decreases with the increase in skinning severity. Severely skinned Chronicle has significantly lower concentrations (P <0.05) in severe category than in intact one, and for Concerto this values are also lower for severe category, than for the intact grains, however this difference is not statistically significant (P >0.05). Fructose, glucose and sucrose were the highest in intact skinning categories and the lowest for severe category. The difference in concentrations between those two categories was significant for variety Chronicle (P <0.05) for fructose, glucose and sucrose. For variety Concerto only the difference in concentration between intact and severe categories in sucrose was significant (P <0.05), and differences between concentrations of glucose and fructose for Concerto were not significant (P >0.05). There was no significant difference (P >0.05) between the variety for the category in concentrations of maltose. Concentration of maltotriose was significantly higher (P <0.05) for severe category, and variety was not statistically significant (P >0.05).

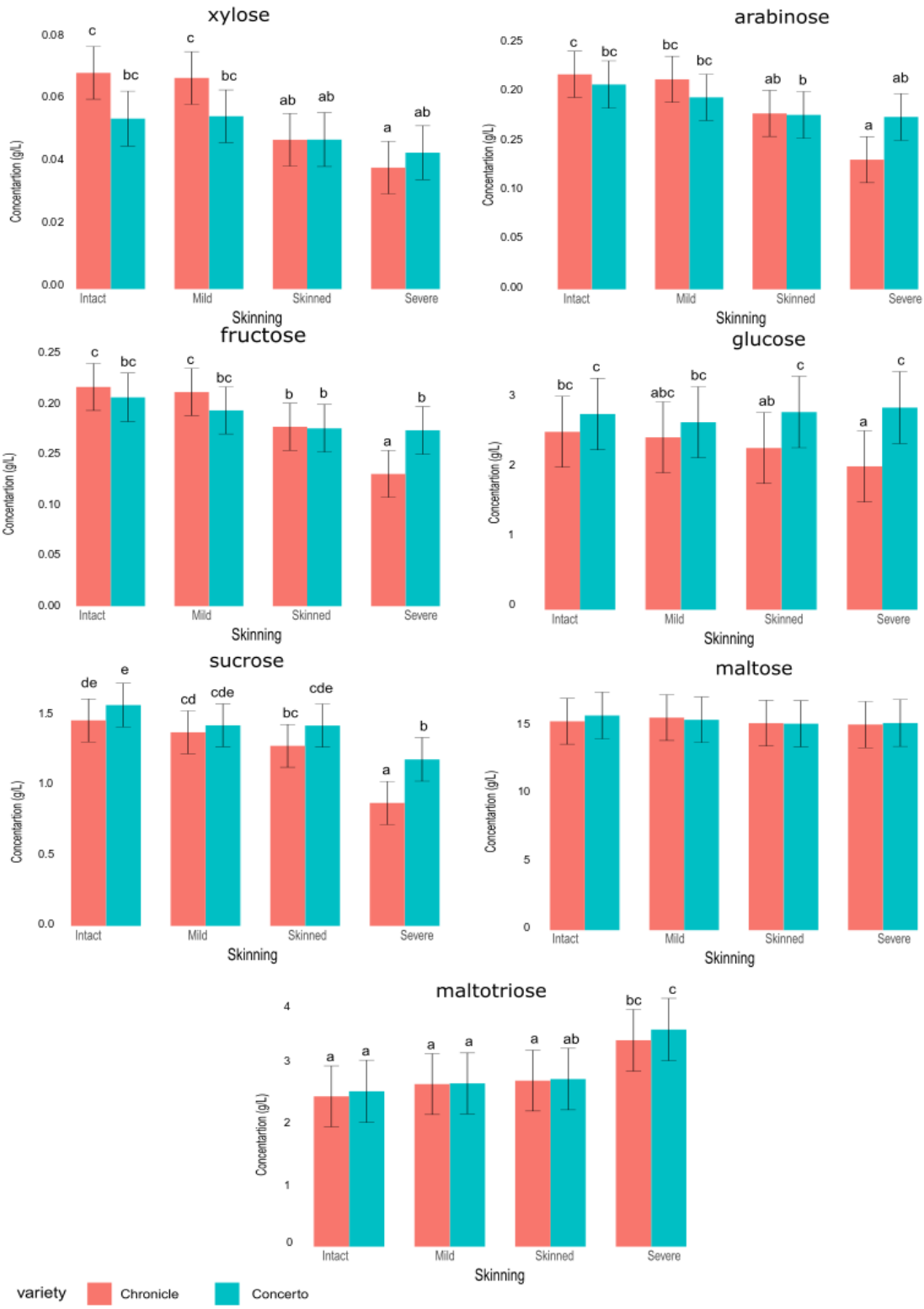


Figure 4.8. Concentrations of mono- and polysaccharides in malt hot water extract for Chronicle and Concerto varieties, with varying skinning levels. Categories and varieties sharing a letter are not significantly different from each other (P >0.05), 95% confidence intervals have been plotted.

4.3.3 Overmodification – Scanning electron microscopy

The interaction between skinning category and scoring criteria of the malted grains was significant (P <0.05). Each of the scoring criteria (A granule pitting, B granule degradation and cell wall breakdown) of malted grains had a significantly lower score than the overmalted grains (P <0.05). Cell wall degradation and B granule degradation were not significantly different for each of the skinning categories; and the only difference was in A granule pitting between skinning categories intact and skinned (P <0.05).

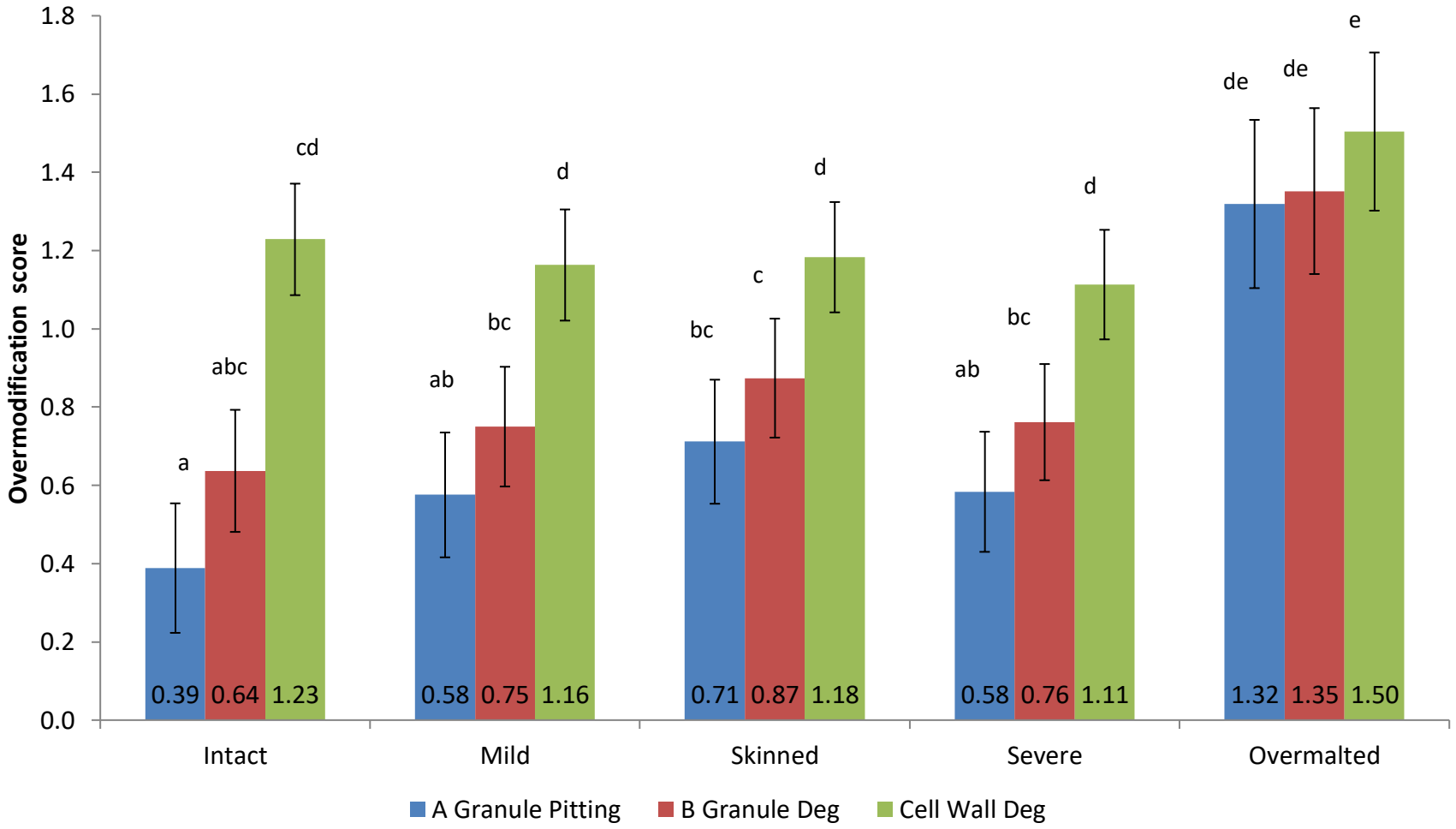


Figure 4.9. Estimated means of the scores of three over-malting criteria (A granule pitting; B granule degradation; cell wall degradation). 95% confidence intervals have been plotted; categories sharing a letter are not significantly different from each other.

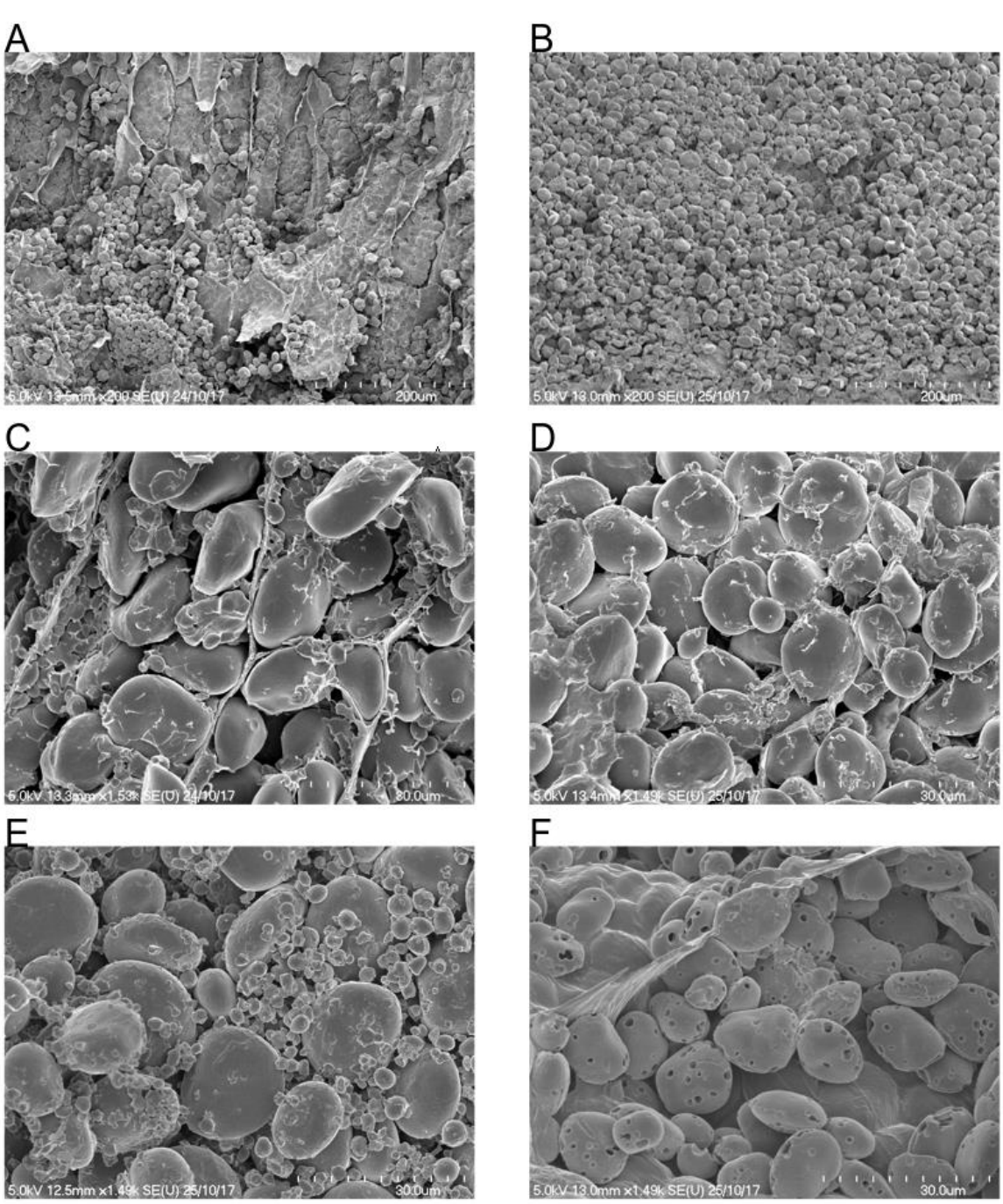


Figure 4.10. Sample images of modification elements scored. A- unmalted grain, with visible cell wall structures; B- malted grain with loose starch granules; C-higher magnification of cell walls in unmalted grains; D-malted grain, lack of visible cell walls and small B starch granules and protein matrix; E- A granules and a large quantities of small B granules in unmalted grain; F – severe A granule pitting in overmalted grains.

4.4 Discussion

Homogeneous modification is the key objective of the malting process. Under- and over-modification is undesirable as this affects the starch availability for conversion into simple sugars and therefore for the yeast to ferment during downstream processes. Results of the experimental work in chapter 3 have indicated that skinning causes inefficiencies during malting (Meredith, 1959), and the aim of the experiments in this chapter were to investigate if this is a result of undermodification or overmodification of the skinned grains. Two contrasting theories attempt to explain the inefficiencies in malting. The first hypothesis suggests that undermodification of the grains takes place, due to lack of germination as a result of excessive embryo damage or halting of the germination due to acrospire damage (Agu et al., 2002; Macleod & Palmer, 1968); the second theory proposes overmodification occurs in skinned grains, as grains without husks take up water faster and therefore germinate faster (Bryce et al., 2010) and removal of the husk increases availability of the oxygen to the embryo (Lenoir et al., 1986).

Undermodification of the grains is routinely tested for in bulks of grains, using a friabilimeter. Friability and homogeneity of the sample are assessed on a 50 g sample of the malted grains and it is a quick, reliable and reproducible method in a commercial setting (Baxter & O'Farrel, 1983; Fox et al., 2001; Giarrantano & Thomas, 1985). Despite its high accuracy, the friabilimeter method does not give any information on the single grain level and does not account for unusually malted grains, including 'case-hardened' malt, which is perfectly modified, but hardened due

to the kilning conditions (Thomas, 1986). Another established method of measuring undermodification is the Carlsberg Calcofluor Modification method, in which calcofluor binds to the unmodified regions of the grain (Aastrup et al., 1981; Aastrup, 1988). The calcofluor method is more laborious than the friabilimeter in assessing the modification, and Giarrantano & Thomas, (1985) suggest that it is less reproducible for grain bulks, which is likely to be due to the fact that the sample assessed by the calcofluor method is much smaller than that in the friabilimeter. However, the calcofluor method gives much more detailed, single grain level information allowing for not only quantification of the undermodification, but also visualisation of the problem areas. Using calcofluor staining it is possible to differentiate between two distinct undermodification issues: whether a sample is undermodified because of large proportion of whole unmodified grains, or large proportion of grains in which only partial modification has occurred. In the calcofluor experiment the results suggested that an increase in skinning results in the increase in unmodified regions of the grains. In addition it is evident that in skinned and severe samples, there is an increase in number of grains that have not modified at all, which is consistent with the theories of Agu et al., (2002) and Macleod & Palmer (1968) that skinned grains have higher proportion of damaged embryos, which do not germinate at all. In addition there is a higher proportion of grains that have only partially modified in skinned and severe samples, suggesting that modification progresses is either slower in the grains that have lost their husk or that it is halted prematurely, due to the damage of the growing acrospire (Agu et al., 2002). Intact category had the lowest proportion of undermodification, although this was not statistically significant from

the mild category, possibly due to a very small difference in the skinning levels. Mild category, as described in chapter 3 was a field category, as it was delivered after harvest, with skinning levels acceptable by industry for malting. This difference between mild and intact categories, although not significant, is of interest because although it may not be significant at the laboratory scale, it suggests that even very low levels of skinning in a large bulk could impact on how modification progresses. This findings confirm observations made by Meredith (1959), who also observed poorer performance of samples containing small proportion of skinned grains.

Overmodification is more difficult to measure, and there are no standard methods in use to assess grains for this parameter, but it can have detrimental effects on the handling of malt and on the beer produced. Overmodified malts break easily in transport and during handling, increasing the necessity for costly dust extraction. Beers produced from overmodified malts are thin in character, they lack body and there are issues with head retention (Briggs, 1998; Herb et al., 2017; Depraetere et al., 2004). Enzymes produced and released during malting hydrolyse the starch into mono and oligosaccharides, and their proportions in the extract vary depending on the degree of modification of the endosperm. Therefore measurement of sugars associated with breakdown of cell walls and starch in the endosperm in the hot water extract informs on both under and overmodification and understanding of the sugars present in the extract allows for prediction of the progress of malting (Chiba et al., 2012; Agu et al., 2016; Otter & Taylor, 1967).

Barley endosperm is composed of starch granules, enclosed in thin cell walls composed in 80% β-glucan and 20% arabinoxylan; which during malting is hydrolysed into arabinose and xylose (Debyser et al., 1997). In malt arabinoxylan is present in low quantities, usually less than 1% of the dry malt (Debyser et al., 1997). The hydrolysis of arabinoxylan to arabinose and xylose during malting results in the increase quantities of these monomers in the malt extract, therefore higher levels of those monosaccharides are indicative of improved cell wall breakdown. Malt with severe skinning had significantly lower quantities of these two monosaccharides than intact category, which indicates that skinning results in grains being undermodified. In addition high levels of arabinoxylan in the wort have been linked to beer haze formation (Coote & Kirsop, 1976) and poor membrane filtration of the wort (Sadosky et al., 2002).

Galactose and rhamnose containing polymers are present in the cell walls of the barley endosperm in very small quantities, usually less than 0.5% (Lazaridou et al., 2008). Therefore it is not unexpected, and similarly to findings of MacLeod et al., (1953), the quantities of galactose and rhamnose were too small to be quantifiable by thin layer chromatography in the present study.

Sucrose is present in the embryo of the unmalted barley grain (Henry, 1988), and unlike other monosaccharides it is not the result of starch breakdown, but it is produced from triglycerides during germination in the aleurone layer (Briggs, 1998). It provides an early source of fuel for the growing embryo, and as germination progresses, sucrose as a source of fuel is substituted by the sugars hydrolysed from

the starch in the endosperm. Adequate levels of sucrose in malt are necessary, as it is one of the fermentable sugars. However, high or low levels of sucrose in malt cannot be clearly interpreted as under- or over-modification and they have to be considered with other mono- and oligo-saccharides. In the present study the levels of sucrose were much lower in severely skinned grains, than in intact category, but on its own this could be interpreted as undermodification – sucrose was not produced by the aleurone layer, or overmodification – sucrose levels were depleted by the growing embryo.

Fructose and glucose are monosaccharides produced during the hydrolysis of the starch. Overmodification would result in higher proportion of these sugars in the malt extract. During fermentation fructose is fermented first followed by glucose, maltose and in small quantities maltotriose (Panchal & Stewart, 1982; Stewart, 1973). In addition glucose is utilised faster and to a higher degree than fructose in the extract (D'Amore et al., 1989). When the levels of fructose and glucose in the wort have fallen below a certain level, the hydrolysis of maltose by yeast can begin (Griffin, 1970). Glucose and fructose are the preferred carbon sources for the yeast, and excess glucose causes repression of the transcription of genes responsible for synthesis of enzymes necessary for the utilization of alternative carbon sources, including maltose (Gancedo, 1998; Meneses et al., 2002). This is called 'carbon catabolite repression', and is detrimental for wort fermenting yeast, as maltose is the most abundant sugar in the wort, and needs to be fully utilised to obtain the full fermentation, with maximised ethanol yield. In this study levels of fructose in both varieties and glucose in variety Chronicle, were much lower for the severely skinned categories than for

the intact category. These low levels of monosaccharides would indicate poor or incomplete modification, and neither the skinned or severe category showed signs of overmodification, where those sugars would be expected to be at high levels. Additionally, maltose levels in all skinning categories were not significantly different, and it would be expected that overmodification would result in lower levels of maltose, as it would have been broken down into glucose.

Maltotriose is a trisaccharide consisting of three glucose molecules. High levels of maltotriose indicate a poorly modified malt, in which incomplete breakdown of the starch occurred (MacLeod et al., 1953). This is usually a result of insufficient levels of enzymes developed during malt modification. Most strains of yeast are unable to ferment maltotriose, or do so in very insufficient quantities. Significantly higher levels of maltotriose in the hot water extracts of malts produced from skinned and severely skinned grains, indicated that skinning causes undermodification, with no signs of overmodification in the grains.

Considering all of the above mono and oligosaccharides it is clear that no sign of overmodification of malt with increased skinning was observed. Conversely undermodification was more evident in the malt with large proportion of grain skinning in the bulk.

The knowledge of the physiological changes during malting made it possible to establish overmodification criteria, which could be visually assessed and scored in malted grains. The first change in grains during malting is breakdown of cell walls in the endosperm followed by the degradation of the outer edge of the starch granules

(Gram, 1982). During malting small starch granules are degraded faster than the large granules (Bathgate & Palmer, 1973; Palmer, 1972a), and large starch granules in the grains show pitting of the surface of the A- granules, due to the action of α-amylase if grain are overmodified (Palmer, 1972a; MacGregor & Ballance, 1980; Kano et al., 1981). After examining and scoring the grains from each of the malting categories on the three criteria of overmodification: cell wall degradation, B granule degradation and A granule pitting, it was evident that there are no signs of overmodification in any of the skinning categories, as all of the categories had lower scores than the positive control.

Experimental work in this chapter aimed at examining two theories of how skinning negatively influences malt modification. The first theory suggested that skinned grains do not modify fully due to damaged embryos and damage of the acrospire during germination (Macleod & Palmer, 1968; Agu et al., 2002). The second hypothesis was that skinned grains germinate faster, leading to overmodification of the malt and loss of valuable extract. Bryce et al., (2010) suggests that it is due to a faster water uptake, that skinned grains germinate faster; Lenoir et al., (1986) hypothesised that polyphenols present in the husk slow down the germination, therefore skinned grains would have overcome this barrier, allowing easier access of the embryo to the oxygen. It was clear from the results of this chapter there was no evidence of overmodification in bulks containing large proportion of skinned grains, and undermodification of the grains was observed in categories with large proportion of skinning. This does not conclusively state that grains without husk never germinate faster. Differences in methods and varieties used mean no overmodification was

observed in current study; however Bryce et al., (2010) used a different micromalting regime and, and Lenoir et al., (1986) used a very small sample of the grains, which did not go through the micromalting process.

4.5 Conclusions

Experimental work in this chapter suggests no sign of overmodification in bulks with large proportions of skinned grains, and it is undermodification that is responsible for poor performance of the malt with skinned grains. It is important for the maltsters to know whether over- or under-modification is occurring, as the solutions for this problem are very different. Undermodified malt can be used in brewing with addition of exogenous enzymes, which would aid in breaking down the starch; whereas overmodified malt would require shorter or lower temperature of the malting regime.

Conclusions from chapter 3 were that skinning causes inefficiencies in malting, and malt produced is of inferior quality. Experimental work in this chapter has clearly indicated that undermodification is the cause of the lower hot water extract, and that friability and homogeneity assessed using friabilimeter were an accurate representation of the malts' levels of modification, and although they give less detailed picture of the modification, they are in agreement with the undermodification results of this chapter.

Chapter 5. Impact of the type of husk-loss on modification

5.1 Introduction

This thesis, so far, has presented skinning as the proportion of grains with more than 1/5 of husk-loss. However different types of husk-loss can occur depending on how the grain sample has been handled. In the field samples, which are combine harvested, the most common type of damage is complete loss of the lemma or the complete loss of both the lemma and palea. In contrast, samples for experiments in chapter 3 were prepared by threshing the grains from combine harvested field samples, in order to exacerbate skinning and further remove the husk. This approach was very useful for the preparation of large samples for micromalting, with quantified damage to the husks. Threshed grains had unevenly broken husks with small proportions usually attached to the grain, even in severe skinning. Combine harvested grains, are more prone to complete loss of lemma or complete loss of both lemma and pale. This difference in skinning was described in Chapter 2, in which two different mechanisms of skinning were identified, depending on the grain moisture during harvest or threshing.

The work in this chapter tests the hypothesis that there are differences between the germination and modification of the grains depending on the type of husk-loss. It is hypothesised that grains with either undamaged husk or huskless

damage will perform better than grains with the lemma missing. Understanding these differences could further explain the inefficiencies in malting of skinned grains and could potentially aid maltsters even further in their decision making in processing of skinned grains. A good example of this would be a sample with low skinning in the bulk, but where of those skinned grains a large proportion was huskless. It is possible that such samples could be more efficiently processed using parameters different to that of bulks with large proportions of grains with a missing lemma for example. In addition, it is important to examine the modification of the grains where it has been verified that the embryos are intact. Undermodification of the grains was evident in the results from chapter 3 and 4; however, the cause of this undermodification was not determined, it was speculated that it is due to damaged embryos or acrospires. Small scale tests with visually undamaged embryos, and where mechanical damage to the acrospire would not occur, gives a better understanding of the impact of the type of damage on the grains and how this could affect the efficiency of the malting.

Observations made in chapter 3 during the germination phase of micromalting, indicated differences in the grain development in different skinning categories. As germination progressed, it became apparent that intact grains had large proportion of rootlets, with no evidence of overgrown acrospires. In the samples with severe skinning, the grains showed little rootlet growth, with a long acrospire. As a visual the reminder, images of this observation are presented again in Figure 5.1.

A

B

Figure 5.1. Grains of Concerto during micromalting, germination day 4. A. intact category B. skinned, with visible differences in root and acrospire growth.

Overgrown acrospires are interpreted as a sign of overmodification by the maltsters, who use a visual method of assessing modification, when the acrospire has reached ¾ of the grain length, the modification is said to be complete, if however the acrospire is longer than the grain length it is thought the grains are overmodified

(Briggs, 1998). The conclusions from chapter 4 were that there is no evidence of overmodification in skinned grains and undermodification is the cause of inefficiencies in malt production. The micromalting experiments were done on relatively large samples (50 g), and it was not possible to examine the growth of individual grains over time in the large bulk.

Previous research on the role the husk plays in germination was often conducted in connection with the effects that husk absence has on grain dormancy. These studies on germination and dormancy have often implicated the coleorhiza; a sheath protecting the root in the embryo of the grain, graphical representation of coleorhiza is presented in Figure 5.2.

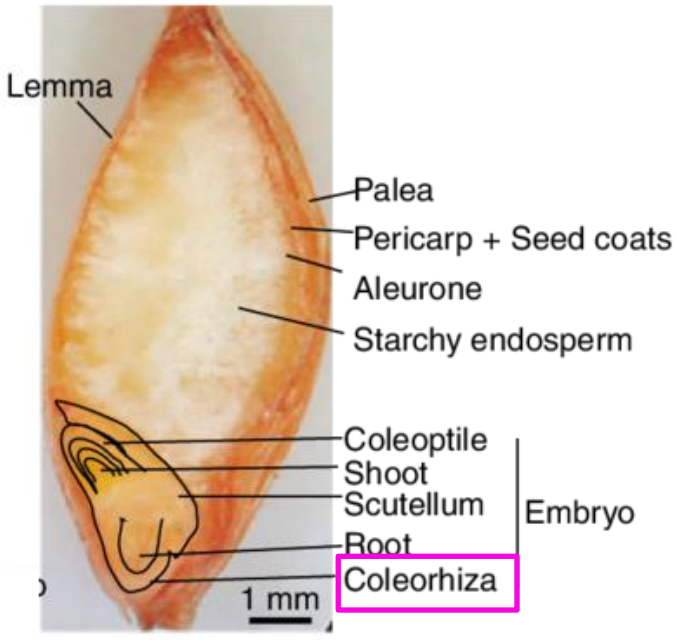


Figure 5.2. Barley grain with coleorhiza source: (Rodríguez et al., 2015)

It was speculated that this tissue is the first to sense the presence of the water, and exposed embryos in grains without husk could therefore sense the water faster, and therefore germinate faster (Davidson, 1979; Sargent & Osborne, 1980). The coleorhiza elongates and separates from the roots during the first 18 h of imbibition in grains that have been after ripened (AR) (this is a period in which grains alleviate dormancy), allowing for the root to push through it. In dormant grains the coleorhiza provides a physical barrier to the root and prevents its emergence (Barrero et al., 2009). Another hypothesis suggests that oxygen availability to the embryo is limited by husk, therefore slowing the germination in intact grains (Lenoir et al., 1986; Benech-Arnold et al., 2006; Bradford et al., 2008). Rodríguez et al., (2015) attributed this to the presence of polyphenols and their effects on dormancy, husks contain large quantities of polyphenols, some of which use up the oxygen dissolved in the water in their own reactions of polyphenol oxidase. Therefore, a missing husk can affect the availability of oxygen and speed of reaction. This was first observed by Lenoir (1986), and the conclusion from his study was that removing the husk almost always improved the germination rate because the husk then does not compete with the embryo for the available oxygen. Benech-Arnold et al., (2006) also confirmed this observation, his work on non-dormant grains suggest that the activity of the enzyme in their husk is at the same level comparable with that of dormant grains.

5.1.1 Aims and objectives

This work in this chapter aimed at investigating the influence of the type of husk-loss on germination and modification. The objectives were: i) to determine whether the rate of water uptake varies between the husk-loss types by measuring the moisture content of the grains over the steeping period; ii) to examine the rate and vigour of germination in barley grains with missing lemma and huskless, by examining the root and acrospire growth over time; iii). to investigate the rate of modification in the grains with the missing lemma and huskless by measuring the levels of α-amylase on each germination day.

5.2 Materials and methods

5.2.1 Grain classification into husk-loss patterns

For all the experiments described below grains of the variety Chronicle were used, with the exception of Water uptake experiment, where both Concerto and Chronicle were used. The grains were hand sorted into three categories based on husk-loss type (attached husk, lemma missing and huskless), pictured in Figure 5.3. Intact grains were visually assessed as having less than 1/5 husk-loss. Lemma missing category were grains that had lost the entirety of the lemma only, with an intact palea adhered to the ventral side of the grains. Huskless grains were defined as grains that had lost both the lemma and palea. During sorting, the skinned and lemma missing grains with

visually intact embryos were chosen, those grains missing an embryo were discarded, as damaged or missing embryo prevents grain from germinating.

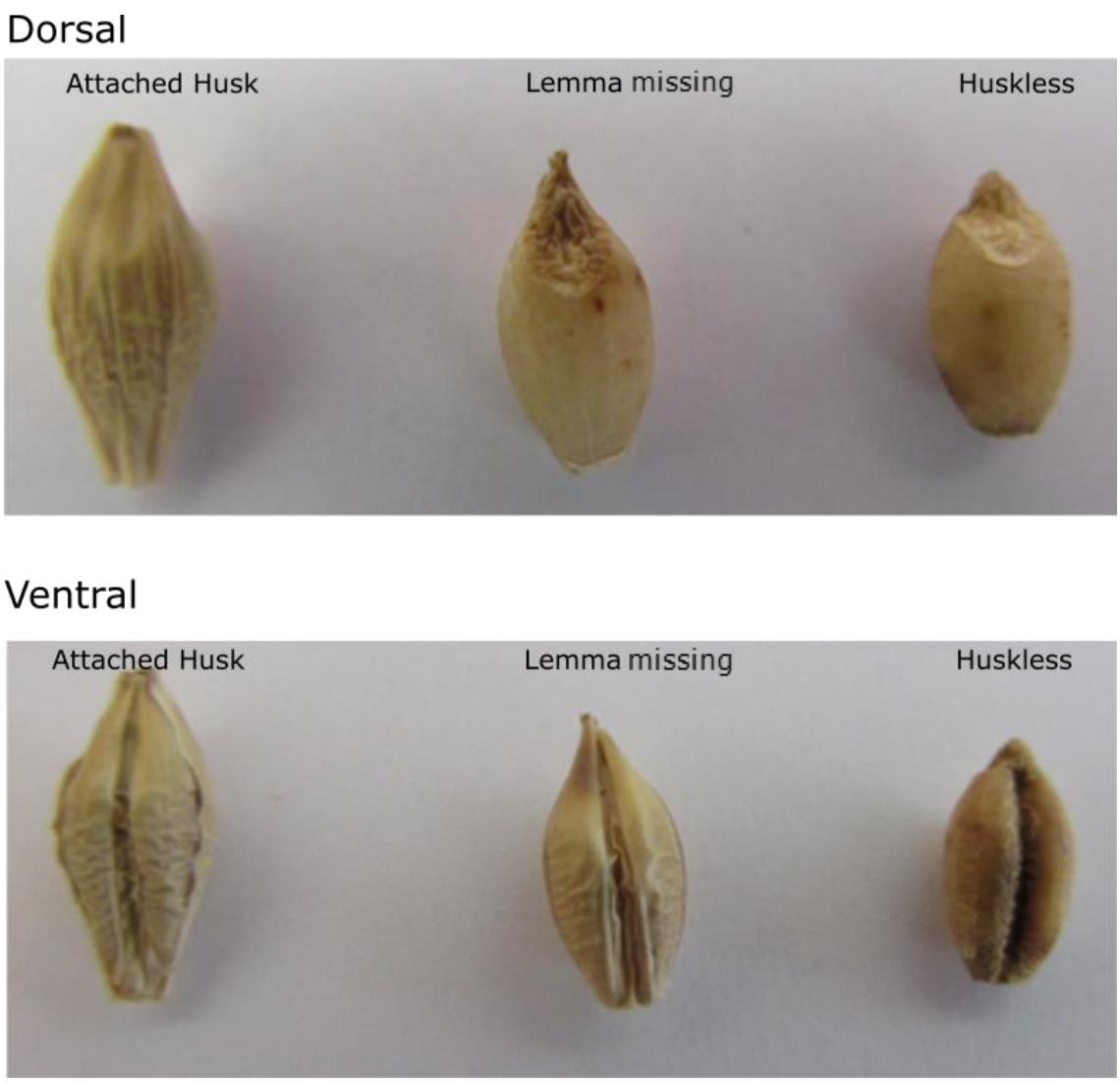


Figure 5.3. Single grain husk-loss types pictured from dorsal and ventral sides (Wood, 2018)

5.2.2 Water uptake

Two spring barley varieties, Concerto and Chronicle, were used. Both varieties have been approved for brewing and distilling. For both varieties, a main stock of 20 kg was sourced from Bairds Malt in Arbroath. From each of the stocks thirty grains of each husk-loss type were selected, these types are presented in Figure 5.3. The categories were established based on most prominent types of damage in grain bulk sourced from the field. Those categories were: 'attached husk', where the husk was intact and tightly attached to the caryopsis; 'lemma missing', with only the lemma of the skinned grain was missing and intact palea was tightly attached to the caryopsis and 'huskless' in which both lemma and palea became detached. Only grains without visible damage to the embryo were selected for this experiment. The experiment was run in triplicate on 10 grains at the time from each husk-loss type. Grains were placed in a tea strainer and fully submerged in tap water at 16^{o}C; the temperature of the water was maintained constant in the incubator set to the same temperature. The weight of the grains was recorded at time intervals: every 60 min for the first 8 h and then at 24 h, 32 h and 48 h. Before weighing the grains the surface water was removed from the grains with a filter paper. An increase in the weight of the grains was indicative of the absorption of the water into the grains and was calculated as moisture content and expressed as percent on wet basis (wb). Final weight measured as weight of the grain was verified by measuring the final moisture content using the method described in section

5.2.3 Germination rate and vigour

Measurements of grain germination rate and vigour in this chapter was conducted by an undergraduate student (Wood, 2018) under my supervision. Two filter papers were placed in each 9 cm Petri dish; to each dish 10 grains of a defined husk-loss type category and 5 ml of water were added. Grains were equally spaced out on the petri dish and positioned ventral side/crease up (embryo/lemma side down). Grains were incubated at 18°C in darkness for one, two, three, four or five days. Following incubation, digital callipers (accuracy: 0.01 mm) were used to measure the length of the grain, acrospire and longest root. To measure acrospire length of intact grains the lemma must be removed, therefore a new set of grains had to be set up for each day.

5.2.4 Grain α-amylase

Measurements of grain α-amylase in this chapter was conducted by an undergraduate student (Wood, 2018) under my supervision.

Plant material

The germinated grains used in the above rate and vigour experiments were used for measurement of the rate of synthesis of the α-amylase in those grains. The extraction of α-amylase was done using Megazyme α-Amylase Assay Kit (Ceralpha Method) (Megazyme, Ireland). The kit included concentrated reagents and instructions on preparation of reagents not included in the kit, or additional concentrated reagents.

Reagents that were supplied in the kit were the following: amylase HR reagent, extraction buffer and stopping reagent. The other reagents were prepared according to the kit instructions.

Extraction of α-amylase

Following the physical measurements, acrospires and roots were removed with a scalpel. Each 10 grain sample was ground thoroughly using a clean pestle mortar. Extraction buffer (1 ml) (Megazyme extraction buffer A) was added to allow grinding to a thick paste. The mixture was transferred to a labelled 15 ml falcon tube with the aid of a disposable spatula. A further 2 ml of extraction buffer was pipetted into the pestle to wash the sides and ensure the plant material was quantitatively transferred to the falcon tube. Tubes were stored on ice while further samples were ground. Following grinding all samples tubes were topped up with buffer to give an equal volume in each tube (5 ml). At this point samples were either prepared and assayed immediately or stored at -20°C for assay on another day.

Enzyme activity assay

Samples were centrifuged for 3 min at 2,000 g and the supernatant transferred to a fresh tube. The expected α-amylase content varied widely depending on the germination time; sample supernatant was therefore diluted with extraction buffer according to Table 5.1. Each of the diluted samples prepared above was assayed in duplicate, providing a technical replicate to mitigate timing errors in the assay procedure. Aliquots of 0.2 ml of Amylase HR Reagent solution (Megazyme, Ireland) were dispensed into test tubes and pre-incubated in a water bath at 40°C for 5 min.

Diluted samples were also pre-incubated at 40°C for 5 min. A 0.2 ml aliquot of diluted sample extract was added to each tube containing Amylase HR Reagent and incubated at 40 °C for exactly 20 min from the time of addition. To optimise timing accuracy aliquots were added at 30 tube contents shaken vigorously. The absorbance of the solutions was read using a spectrophotometer at 400 nm (Agilent 8453 UV-visible Spectroscopy System, Agilent Technologies, Santa Clara, California USA) against the blank, which was created by adding the Amylase HR Reagent aliquots (0.2 ml) and malt extract solutions (0.2 ml) directly to the 3 ml of stopping reagent without allowing for any development time.

Table 5.1. Dilution of experimental samples according to day to adjust for range of enzyme concentrations in original sample

Germination stage	Volume of extract	Volume of buffer	Dilution factor
Day 1	500µl	3500µl	8
Day 2	250µl	3750µl	16
Day 3	50µl	3950µl	80
Day 4	10µl	3990µl	400
Day 5	5µl	3995µl	800

Calculation of α-amylase activity

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one micromole of *P*-nitrophenol from BPNPG7 in one minute under the defined assay conditions, and is termed a Ceralpha Unit (McCleary et al., 2002; McCleary & Sheehan, 1987).

Units/g Flour:

Equation 9. Ceralpha units in grains

$$\frac{\Delta E_{400}}{Incubation\ Time} x \frac{Total\ Volume \in Cell}{Aliquot\ Assayed} x \frac{1}{\varepsilon_{mM}} x \frac{Extraction\ Vol}{Number\ of\ Grains} x\ Dilution$$

where:

ΔE_{400} = Absorbance (reaction) - Absorbance (blank)
$Incubation\ Time$ = 20 min
$Total\ Volume\ in\ Cell$ = 3.4 ml
$Aliquot\ Assayed$ = 0.2 ml
εmM of *P*-nitrophenol (at 400 nm) in 1% tri-sodium phosphate = 18.1
$Extraction\ volume$ = 5 ml
$Dilution$ = Dilution of the original extract (depending on germination day: 8, 16, 80, 400, 800)

For grains this is:

Equation 10. Ceralpha units in grains

$$\frac{\Delta E_{400}}{20} x \frac{3.4}{0.2} x \frac{1}{18.1} x \frac{5}{10} x20 = \Delta E_{400} x\ 0.02348\ x\ Dilution(8, 16, 80, 400, 800)$$

5.2.5 Statistical analysis

Statistical analyses were conducted using R (R Core Team 2016) and linear mixed effects models were used to determine the effects of the dependent variables and 'day' and 'skinning' and their interaction as the fixed effects, and 'year' and 'rep', as

random effects on each of the Response variables: 'α-amylase', 'root growth', and 'acrospire : grain length' and 'water uptake' using the R package 'lme4' (Bates et al., 2015). Grains with no growth were included in the analysis and the length of acrospire and root was recorded as zero. The minimally adequate models were chosen by comparing hierarchical models using ANOVA (α = 0.05) and significant differences among the effects were determined by least squares means pairwise comparisons using package 'lsmeans' (Lenth, 2016).

5.3 Results

5.3.1 Water uptake

Grains from the two types of husk-loss: lemma missing and huskless, had the mean control moisture content higher than the attached husk category, however within the first one hour of steeping, grains from the attached husk category increased their moisture content to 24%, compared with 22% for lemma and 21.5% for huskless; mean results of the water uptake experiment are presented in Table 5.2 Table 5.2. Mean data of the moisture content (%) ± standard deviation of the skinned barley categories at serial time points. Attached husk grains continued to have the highest moisture content until 8 h of steeping. At 24 h, the MC of grains with missing lemma is the highest, followed by attached husk with the lowest moisture content for naked grains. This is the same for the MC at 32 h and final measurement at 48 h grains with missing lemma have still the highest MC of 46.6%, however huskless grains at this

point have very slightly higher MC than the attached husk grains, 45.88% and 45.75% respectively.

Table 5.2. Mean data of the moisture content (%) ± standard deviation of the skinned barley categories at serial time points.

		Mean MC (%) ± SD		
Concerto	Time (h)	Attached husk	Lemma missing	Huskless
	0	13.19 ± .74	13.47 ± .50	14.49 ± .24
	1	24.69 ± 1.33	21.48 ± .99	22.03 ± .49
	2	27.11 ± .78	24.23 ± 1.19	25.40 ± .60
	3	30.16 ± .79	26.93 ± .60	27.91 ± 1.16
	4	31.23 ± .77	25.98 ± 3.80	29.98 ± 1.10
	5	32.36 ± 1.24	29.78 ± .88	31.43 ± 1.17
	6	33.64 ± .55	31.37 ± .80	32.98 ± 1.03
	7	35.03 ± .79	32.23 ± .78	33.93 ± 1.18
	8	35.79 ± .79	33.22 ± 1.27	34.91 ± .79
	24	38.12 ± 6.06	38.25 ± 2.50	35.42 ± 7.14
	32	43.06 ± 1.46	42.98 ± .31	42.25 ± 2.74
	48	45.60 ± .91	45.84 ± .20	45.86 ± 1.28
Chronicle	Time (h)	Attached husk	Lemma missing	Huskless
	0	12.32 ± 1.78	14.22 ± .20	13.25 ± .87
	1	23.62 ± 1.34	22.71 ± .79	20.98 ± 1.15
	2	27.65 ± 2.43	25.51 ± .23	24.61 ± .73
	3	29.41 ± 2.62	28.29 ± .51	26.92 ± .52
	4	30.97 ± 1.67	30.60 ± .53	28.86 ± .79
	5	32.43 ± 1.65	32.15 ± .53	30.72 ± .63
	6	33.39 ± 1.87	33.23 ± .49	31.84 ± .55
	7	34.82 ± 2.18	34.73 ± .93	33.19 ± .71
	8	35.92 ± 1.45	35.23 ± .64	33.85 ± .58
	24	37.70 ± 4.19	41.35 ± 2.60	35.54 ± 7.65
	32	43.20 ± 1.46	44.61 ± 1.12	41.90 ± 2.68
	48	45.90 ± 1.70	47.36 ± .20	45.89 ± 1.44

The interaction of the husk-loss type and variety were significant (P <0.05), **Error! Reference source not found.** presents model output data of the time of steeping on the moisture content. The difference between the types of husk loss in each variety were not statistically significant (P >0.05). Barley grains increase in moisture content at the fastest rate within the first one hour of steeping, after which the increase continuous at a steady rate. Over the 48 h, moisture content increases from approximately 13% to 46%. Variety Concerto showed the slowest rate of water uptake in grains with lemma missing and the fastest in huskless grains. In contrast, the variety Chronicle had the lowest MCs in attached husk grains and the highest MCs in grains with missing lemma.

5.3.2 Germination rate and vigour

The lowest germination rate was found in the grains with lemma missing. The shortest roots were also measured in grains with the lemma missing and the longest roots were found in attached husk grains, the means and standard deviations are presented in Table 5.3. On day five of germination, the acrospire in proportion to the length of the grain was again the lowest for lemma missing grains; however, the highest was measured in huskless grains.

Table 5.3.Mean root length (mm) ± SD and the proportion of acrospire : grain length ± SD

Root length (mm)			
Day	**Attached husk**	**Lemma Missing**	**Huskless**
1	1.18 ± 0.50	1.21 ± 0.83	0.93 ± 0.58
2	5.01 ± 3.92	8.33 ± 6.50	5.64 ± 5.22
3	17.45 ± 8.79	21.09 ± 13.56	14.82 ± 13.32
4	34.86 ± 16.06	36.00 ± 24.17	26.21 ± 23.22
5	58.15 ± 20.33	55.05 ± 24.02	30.76 ± 29.24
Acrospire : grain proportion			
1	0.15 ± 0.04	0.18 ± 0.07	0.19 ± 0.11
2	0.34 ± 0.15	0.56 ± 0.20	0.50 ± 0.27
3	0.88 ± 0.39	1.18 ± 0.56	1.12 ± 0.66
4	1.99 ± 0.83	2.49 ± 1.46	2.24 ± 1.61
5	3.93 ± 1.26	4.20 ± 1.61	3.23 ± 2.32

The most vigorous root growth was observed for attached husk and lemma missing grains, the difference between the two categories was not significant (P >0.05). The interaction of husk loss type and day of germination was significant (P <0.05). Huskless grains showed significantly slower root growth rate than lemma missing and attached husk grains (P <0.001), Figure 5.4 shows the regressions for root growth (mm) over 5 days, for the three husk-loss types. The rate of root growth for the grains with attached husk was the fastest at 14.11 mm each day, followed by the grains with missing lemma with the rate of root growth of 13.32 mm per day, and the slowest rate of growth was observed for the huskless grains at 7.83 mm per day.

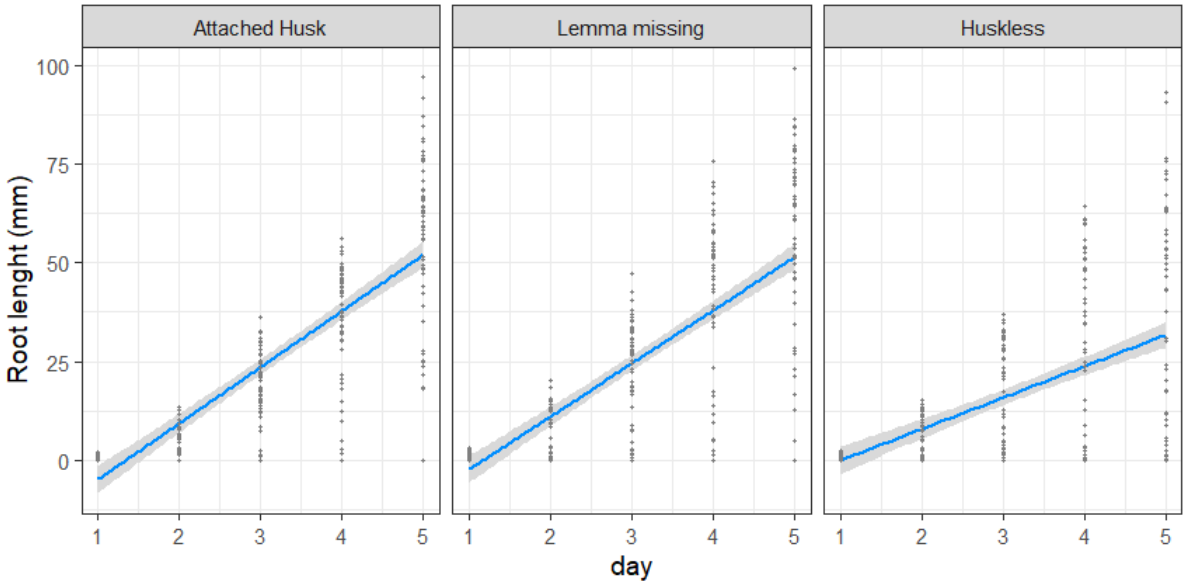


Figure 5.4. Regression of root growth (mm) over five days of germination, for three types of husk-loss. Grey ribbons represent 95% confidence intervals.

The vigour of acrospire growth measured as a proportion of acrospire to grain length was not significantly different for huskless and attached husk categories (*P* >0.05). The fastest growth and the highest proportion of acrospire: grain length was observed for lemma missing category, which was significantly higher than for attached husk or huskless categories (*P* <0.001), Figure 5.5 shows the regression of acrospire growth in proportion to grain length over five days of germination. The proportion of the acrospire length to grain length increases in grains with attached husk increases at a rate of 0.903 per day, in grain with missing lemma it increases by 0.983 each day and it is the slowest for the huskless grains at 0.767 a day.

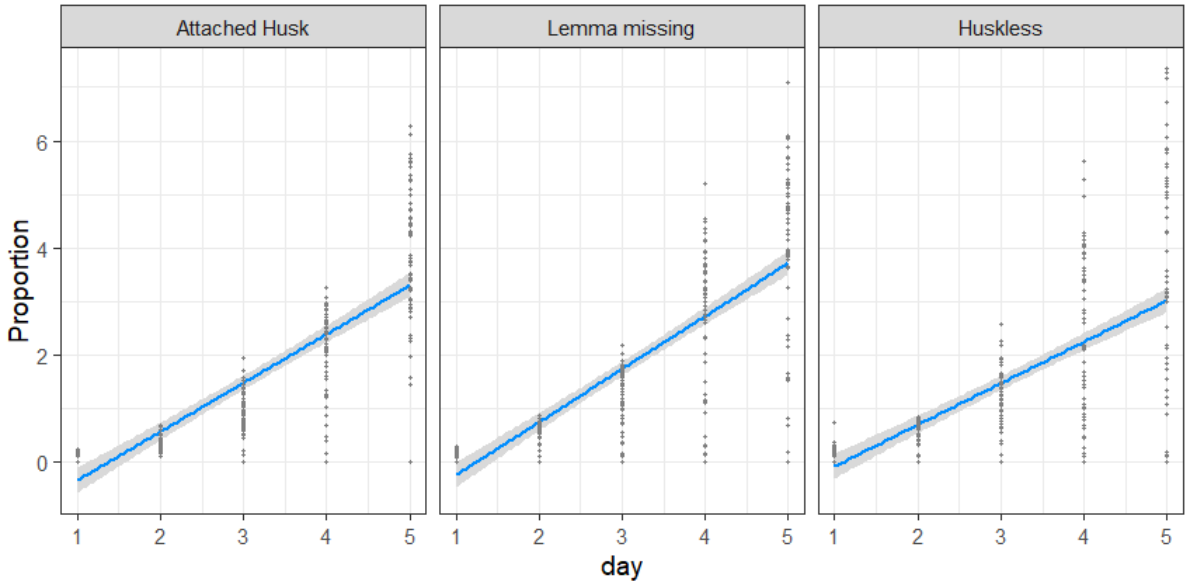


Figure 5.5. Regression of the acrospire growth in proportion to the grain length during five days of germination. Grey ribbons represent 95% confidence intervals.

5.3.3 Grain α-amylase

Mean results of the concentration of α-amylase for each germination day are presented in Table 5.4.

Table 5.4. Mean ± SD of α- amylase production on days one to five of germination in grains with different husk-loss types.

	Mean Concentration (CU/grain) ± SD		
Day	Attached husk	Lemma missing	Huskless
1	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.08
2	0.15 ± 0.04	0.29 ± 0.13	0.00 ± 0.01
3	1.26 ± 0.70	1.46 ± 0.90	1.01 ± 0.53
4	2.89 ± 1.17	3.55 ± 0.82	2.14 ± 1.36
5	3.26 ± 1.43	4.15 ± 2.32	2.12 ± 0.89

The interaction of husk-loss type and day of germination had a significant effect on grain α-amylase content (P <0.05). Huskless grains produced significantly lower

quantity of α-amylase than lemma missing grains (P <0.01), which had the highest concentrations of α-amylase. The difference in α-amylase production between attached husk and lemma missing and attached husk and huskless grains was not significantly different (P >0.05). Lemma missing and huskless grains produced significantly different (P <0.05) levels of α-amylase. Total α-amylase produced by the germinating grains on day five was the highest for lemma missing category and the lowest for huskless category. The rate of α-amylase production for grains with attached husk was 0.9197 CU/grain each day, for grains with lemma missing 1.15 CU/grain and it was the lowest for grains with missing husk at 0.6193 CU/grain each day.

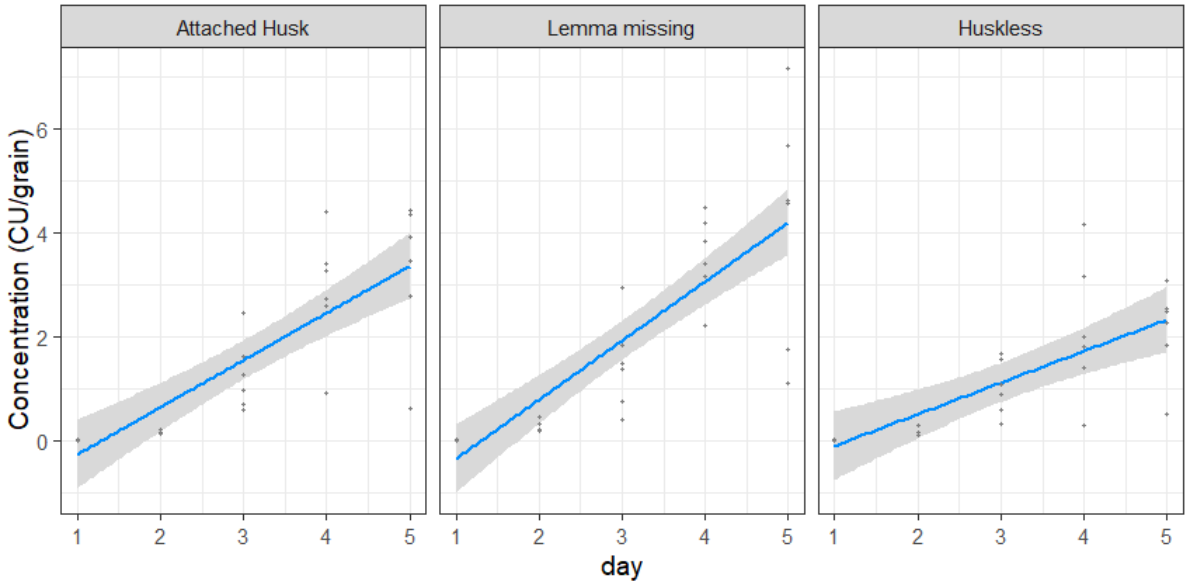


Figure 5.6 . α-Amylase concentration in grains with three different husk-loss types over the five day germination. Grey ribbons represent 95% confidence intervals.

5.3.4 Summary results

Results of each experiment in this chapter were classified in to one of three categories: slow, intermediate and fast, depending on the rate of water uptake, germination and α-amylase production and it is presented in Table 5.5. This visual representation makes it clear that the rate of water uptake was reflected in the rate of germination or production of α-amylase. In addition, the rate and vigour of germination and production of α-amylase showed the same pattern only for huskless grains, which germinated slowly and produced α-amylase slowly. Grains with lemma missing and attached husk did not follow this pattern; lemma missing grains produced α-amylase at faster rate than grains with attached husk.

Table 5.5 Summary results of the experimental work in this chapter. Rate of water uptake, growth and α-amylase production were classified into three categories: slow, intermediate and fast for each husk-loss type.

	Water uptake Concerto	Water uptake Chronicle	Germination root growth	Germination acrospire	Germination α-amylase
Attached husk	Intermediate	Slow	Fast	Fast	Intermediate
Lemma missing	Slow	Fast	Intermediate	Intermediate	Fast
Huskless	Fast	Intermediate	Slow	Slow	Slow

5.4 Discussion

Homogeneity is a key aspect throughout the process of malt production. Maltsters aim to produce evenly modified malt, with as little heterogeneity as possible. Heterogeneously modified malt causes loss of extract, which would be fermented by yeast into ethanol. Production of homogeneous malt starts with barley grains, and previous chapters have concluded that skinning causes inefficiency in malt production due to large proportion of undermodified grains. This chapter aimed at investigating more precisely how different types of skinning affect the water uptake, germination and α-amylase production of the grains to determine whether this could potentially have an impact on the quality of malt produced. Small scale experimental work in this chapter allowed for exclusion of damaged embryos and mechanical damage to the growing acrospire as a cause of the inefficiencies in barley modification.

Barley grains imbibe water through the micropyle in the embryo of the grains and it is then distributed through the grain tissues (Evers & Millar, 2002). The coleorhiza in the embryo has been speculated to be the first tissue to sense the presence of the water and to take part in the initiation of germination; the husk has been described as additional barrier in the uptake of water into the grain, and therefore its removal would be expected to accelerate the initiation of germination (Davidson, 1979; Sargent & Osborne, 1980). Lenoir et al., (1986) and Benech-Arnold et al., (2006), suggest that activity of the polyphenol oxidase in the husk competes with the embryo for the available oxygen, and removal of the husk in dormant grains results in alleviation of dormancy and germination of grains. Dunwell, (1981) achieved a similar removal of dormancy upon detachment of husk in barley grains, but he has concluded that this is due to balance of the hormones promoting and halting the germination, and due to physical barrier of husk presence. In accordance with these theories it would be expected that huskless grains will germinate the fastest followed by lemma missing and attached husk grains. The results presented in this chapter show that this is not the case, and in fact grains missing their husks have the slowest rate of germination.

The rate of water uptake was inconsistent between the barley varieties, and between the types of skinning, and it cannot be definitively linked to faster germination rates. The variety Chronicle was the fastest to take up water for lemma missing grains followed by huskless and attached husk grains. In germination rate and vigour experiments on the same variety it was fastest for lemma missing followed by attached husk and huskless grains. The two patterns do not match; therefore it

cannot be conclusively determined that faster water uptake is the cause of the faster germination observed. In addition, the variety Concerto behaved very differently in the water uptake experiments and the fastest water uptake was recorded for huskless grains, followed by attached husk and lemma missing grains. Barley grains of different varieties and grown in different environments have different rates of water uptake (Molina-Cano et al., 2002; McEntyre et al., 1998; Swanston et al., 2006). This can often be attributed to differences in the structure of the endosperm (Brookes et al., 1976). Cozzolino et al., (2015) demonstrated that whole barley grains, separated husks and endosperm take up water at different rates, which could account for the differences in water uptake between the different husk-loss types seen in the results of present study. Unfortunately due to limited time and resources, no germination rate and vigour experiments could be carried out on Concerto grains. In order to conclusively link water uptake and germination more work needs to be undertaken on different varieties with various types of husk-loss. It would also be beneficial to understand the water movement into the grain in the different husk-loss types. Non-destructive methods including near infrared reflectance spectroscopy and mid infrared spectroscopy have been previously used in monitoring whole grain barley hydration (Cozzolino et al., 2014; Gordon et al., 2019; Cozzolino et al., 2015; Roumeliotis & Barr, A., 2004).

The type of husk-loss had a significant impact on the rate of root and acrospire growth. Lemma missing grains were germinating much faster and huskless grains were the slowest to germinate, the roots and acrospires developed were shorter compared to grains with lemma missing. There was no visual damage to the huskless

grains; however micro abrasion to the fruit coat of the caryopsis cannot be excluded as the cause of slow development of huskless grains, and this could have potentially been responsible for the slow germination vigour observed. In addition to protecting grains from mechanical damage, husks potentially protect the grains from effects of aging, which include lipid peroxidation, inactivation of the enzymes, disruption of membranes, and damage to DNA (Kong et al., 2015; Hu et al., 2012). These changes are attributed to accumulation of reactive oxygen species (ROS), accumulation of hydrogen peroxide and superoxide radicle have been hypothesised to be the main cause of the oxidative stress, resulting in reduced germination rate in grains (Span et al., 2011; Lehner et al., 2008); seed germination can only occur if the ROS levels are below a certain threshold (Bailly et al., 2008).

Production of enzymes in barley is crucial during malting, and it is a measure of malt quality (Briggs, 1998, 1978; Wentz et al., 2014). In the current work production of α-amylase was of interest, as this enzyme is synthesized *de novo* in germinating barley grains and it is well correlated with malt quality (Duke & Henson, 2008), but the production of this enzyme can also be affected by the conditions during malting (Kleinwächter et al., 2014). Therefore it was a good indicator of the quality of barley samples of the same cultivar, with different types of husk-loss. Similarly to germination rate and vigour, the production of α-amylase is significantly higher in grains with lemma missing, than attached husk or huskless grains, suggesting that these grains do not produce enzymes as effectively. There could be several causes of this such as damage to the embryo or the fruit layers of the caryopsis, not detectable by visual examination. Grain aging in huskless grains may

also occur faster than in undamaged grains, impacting on the rate of enzyme production (Span et al., 2011). α-Amylase in germinating barley grains is produced in the aleurone layer, and it is triggered by the diffusion of gibberellic acid through this layer (Briggs, 1963) and damage of this layer would result in the poor diffusion of GA and reduced production of α-amylase (Schuurink et al., 1992). In addition, individual grains of the same batch of malt can vary up to twofold in their content of α-amylase (Kleinwächter et al., 2014). However the micromalting sample used by Kleinwächter et al., (2014) project was 150 g, which introduces the same issues of heterogeneity as discussed above in relation micromalting of samples from this project in chapter 3.

Lemma missing grains in this study performed the best in the germination rate and vigour and α-amylase activity experiments, and the results of water uptake experiments were inconclusive, suggesting that partial presence of husk was advantageous for both germination vigour and enzyme activity compared to a fully intact or fully missing husk. It is likely that it offered protection from mechanical and aging damage to the fruit layers surrounding caryopsis (Span et al., 2011; Briggs, 1963). Lemma missing grains had the exposed embryos and it is possible that the water uptake, which was slightly faster in variety Chronicle, was the cause of faster germination. Dunwell, (1981) and Lenoir et al., (1986) have both shown that removal of husk alleviated dormancy in grain, allowing them to germinate, therefore presence of husk plays a role in inducing and maintain the dormancy and preventing germination. Lenoir et al., (1986) hypothesized that this was due to polyphenol oxidase activity, competing with embryo for available oxygen. Dunwell, (1981)

suggests that inhibition of the germination in husked grains is attributed to hormonal activity and balance of gibberellic acid (GA) and abscisic acid (ABA), and exogenous GA overcomes the dormancy induced by addition of ABA. In husked grains acrospire normally grows under the husk, and although this has never been proven experimentally, it is possible that tightly adhering husk slows down the growth of acrospire under the husk, the factor which has been removed from the lemma missing grains, allowing them for faster growth of the acrospire. It has been shown in various studies that interactions between structures of barley grains are complex and not well understood, including the role of scutellum (Radley, 1969), husk (Lenoir et al., 1986; Dunwell, 1981) or aleurone (Schuurink et al., 1992); they interact, either promoting or suppressing germination.

5.5 Conclusions

Type of husk-loss plays an important role in barley grain germination rate and vigour and in α-amylase production. Grains with missing lemma germinate the fastest and produce the highest levels of α-amylase, second best are attached husk grains followed by huskless grains. This cannot be explained by faster water uptake in those grains, as the water uptake patterns do not match those of germination and α-amylase, and the two varieties examined had different rates of water uptake. Further experimental work could potentially link them more conclusively; grain anatomy should also be explored with respect to water uptake and germination. Grains with lemma missing appear to have the 'best of both worlds' situation, in which presence

of a proportion of the husk protects them from damage, but lack of the part of the husk also allows grains to germinate faster.

Chapter 6. General discussion

6.1 Implications of the two different physical mechanisms of skinning for research and industry

The observation of two different mechanisms of skinning presented in this thesis confirms previous findings that both genetic and environmental conditions play a role in skinning (Aidun et al., 1990; Froment & South, 2003; Brennan et al., 2017b). Barley grown in the glasshouse is dry and most commonly displays the 'parenchyma cells' skinning mechanism, in which parenchyma cells of the husk break. Barley grown in the field is exposed to wetter growing conditions and the most common skinning mechanism observed in that environment was 'cementing layer', in which the cementing layer is weakened resulting in lack of adhesion. Existence of these two skinning mechanisms further supports the previous findings that even the most resistant varieties could skin if the environmental conditions are not right, especially in high rainfall and/or very dry conditions. Increased moisture weakens the cementing layer between the husk and the caryopsis, whereas dry grains show an increase in breakage of parenchyma cells of the husk, which are in immediate contact with the cementing layer.

The results from short soaking experiments in this thesis clearly showed that grains with very low moisture content skin and short soaking in water improved the

skinning severities. Similar results were seen in grains that were not fully dry and the cells had retained small proportion of moisture from the plant development. This small amount of moisture in very dry grains makes the cells more flexible and prevents the breakage (Lewicki & Jakubczyk, 2004; Lewicki, 1998). It is worth noting that very low levels of moisture in the dry, glasshouse grown grains are rarely seen in the field. In UK, especially in Scotland, it is more common to harvest grains at high moisture contents. However with the changing climate and record breaking increases in temperature, particularly in south of UK and the rest of Europe, this type of skinning mechanism might become more prominent in the field situation. 'Cementing layer' skinning mechanism is observed in the field samples, where high moisture content of the grains was predominant. This type of skinning mechanism is characterised by weak adhesive layer between the husk and the pericarp; this could be a result of the cementing layer being displaced by water. This displacement is why most adhesives will lose their properties in moist or humid conditions (Ebnesajjad & Landrock, 2009; Ebnesajjad, 2009). More specifically to barley, Brennan et al., (2019) found that exposure of the grains to misting during grain development affects the composition of cementing layer, which could be responsible for the weakening of the bond. Second possible explanation for weak adhesion of the grains developing in high moisture conditions could be due to incomplete development and maturation of the cementing layer. This could be influenced by high rainfall during the maturation and result in poor adhesion.

Brennan et al. (2017b) carried out the screening of 200 barley varieties to determine their susceptibility to skinning; all the varieties were grown in the

glasshouse. Data from this thesis would suggest that these grains most likely displayed the breakage of parenchyma skinning mechanism. Therefore an important question needs to be asked: Are the glasshouse results relevant to the field situation? This thesis also begins to answer this. One of the main findings of Brennan et al. (2017b) research was that modern varieties are more prone to skinning than some of the older varieties. Even though the mechanism of skinning might be different to that observed in the field, findings of Brennan et al., (2017b) are so far aligned with what is observed in the field. The varieties which overlap between the glasshouse research and the field experiments are consistently categorised according to their susceptibility, and this classification has been true in both environments. Good examples are varieties Propino and Concerto, which consistently show severe skinning susceptibility in the glasshouse and in the field. In contrast variety Westminster is consistently moderate in both environments. This overlap suggests that potentially there is an overarching, possibly genetic component is responsible for these two different mechanisms of skinning. Therefore the answer to the above question is that the results from glasshouse studies are very important and they do translate to the field situation. The research on barley varieties grown in the glasshouse, including Brennan et al. (2017a, 2017b, 2016) or Hoad et al. (2016) is crucial in explaining skinning and the data from this thesis shows that their findings are relevant to the field situation. Testing new varieties in the glasshouse is often the most efficient way of examining them, although future research should take into consideration that these two separate skinning mechanisms exist. It is possible that breeding programmes in their aspiration for the highest extract are breeding in two

different skinning mechanisms, both of which result in lowered proportion of husk in the bulk. This gives an impression of increased extract recovery from malt produced from those grains, but in fact this inflation is caused by increased proportion of endosperm in the sample.

The extremes of moisture range result in increased skinning severity. In this study the origin of the moisture (developmental or environmental) was not important, both short soaking and developmental moisture have improved skinning. Grains harvested before full ripeness and dry grains exposed to short soaking were protected from skinning by the small amounts of moisture. Moisture content does not describe the hydration of each individual tissue, and in skinning mechanism this might be more important, as different barley tissues hydrate at different rates (Cozzolino et al., 2015). The behaviour of parenchyma cells during drying has been studied in apples, as the edible part of the apple is mainly made of those cells (Lewicki & Pawlak, 2003; Hills & Remigereau, 1997). Drying of the parenchyma cells causes change in the cell size and shape, collapse of the cell structure, disruption of cell walls and separation of the cells from each other (Lewicki & Drzewucka, 1998; Angersbach et al., 1999). This explains why these cells are weak in dry cells. Short soaking of the dry grains in the glasshouse improved the skinning, most likely because moisture makes dry cells elastic (Lewicki, 1998; Lewicki & Jakubczyk, 2004).

This thesis provides a good starting point into research on those levels of moisture which protect grains from skinning. It has established that low and high moisture contents exacerbate skinning. Next step would be to investigate the exact

cut off points at which the role of the moisture changes from protective to detrimental, and whether those levels of moisture correspond to the change in the skinning mechanism. Establishing such points would provide clarity for farmers during harvest and aid them in producing the highest quality barley possible.

6.2 Skinning affects germination in a manner that can impact malt quality

Water uptake into the barley grain, is important for germination and therefore malting. Grains imbibe water through the micropyle in the embryo of the grain, which is the distributed through the grain endosperm. The husk poses a barrier to the water entering the embryo, and therefore intact grains could potentially imbibe water at slower rates than grains without husk. Lenoir et al. (1986) and Dunwell (1981) confirmed that removal of husk resulted in faster germination and alleviation of dormancy. Breeders select new varieties with low dormancy, which do not have to be stored for excessive periods of time before malting in order to break the dormancy. Selecting for such low dormancy varieties could mean the breeders inadvertently select grins susceptible to skinning, as studies described above have clearly shown that removal of husk alleviates dormancy. Agu et al., (2008) and Swanston & Middlefell-Williams (2012) suggested that altered malting regimes in grains without husks were necessary to accommodate faster water uptake and prevent grains failing to germinate and eventually dying.

In this thesis experimental work on water uptake was inconclusive to whether it is in any way linked with faster or slower germination. Some reason for this lack of conclusive evidence was the two varieties tested (Concerto and Chronicle) had different rates of water uptake, but only one of the varieties (Chronicle) was further tested for the germination rate and enzyme production. In addition this particular variety had slow water uptake in grains with attached husk, but fast germination and intermediate production of α-amylase. In grains with missing lemma water uptake was fast, but germination rate was intermediate and α-amylase production was fast.

Verification of the exact role of the rate of water uptake in barley grains would help in understanding the role of mechanism of skinning as well as the type of husk-loss in grains on imbibition. It is possible that mechanisms other than water uptake are responsible for the differences in germination observed in this thesis; some of those mechanisms could include embryo and caryopsis damage during harvesting (Bourgeois, 1993; Bourgeois et al., 1996) or grain ageing (Lehner et al., 2008). Using non-destructive methods would allow for analysis of the imbibition over time, especially distribution of moisture in the grain tissues. Such methods were described previously by Cozzolino et al., (2015, 2013), who used near infrared spectroscopy and mid infrared spectroscopy to monitor the rate of the water uptake in barley in a non-destructive way. Cozzolino et al., (2015) also observed that husks take up water at different rates and produce a different mid infrared spectra; therefore employing those techniques in skinned grains would have to be done with caution. The effects of skinning on the quality of malt produced were quantified in this thesis. It would be beneficial to examine the quality of malt produced from a wider variety of naturally

occurring skinning levels. As a starting point, this thesis examined the extreme samples of skinned barley, but it would be worth investigating less severe samples obtained from a field samples, rather than those manipulated with a thresher. Threshing dry grains is likely to produce parenchyma cell skinning mechanism, which produces sample with mixed skinning mechanisms. This adds complexity to the interpretation of the results, as it is not yet known whether skinning mechanism has an influence on water uptake and malt modification.

Two different mechanisms of skinning could potentially play a role in the water imbibition. Dry grains with broken parenchyma cells loose lose their husk unevenly; it usually fragments with small bits still attached to the caryopsis. These fragments of the husk could possibly obstruct the movement of water between grains. Cozzolino et al., (2015) concluded that husks on their own take up water at different rates than whole endosperm. Husks also play a role in stopping or slowing down the germination process (Lenoir et al., 1986; Dunwell, 1981). It is possible that bits of husk perform polyphenol oxidation and hydration processes in the same way a whole husk would, which even in small proportion could alter the germination rates. In addition, dry grains imbibe water at faster rates (Lewicki, 1998), and as explained previously 'parenchyma cells' skinning mechanism occurs mostly in grains which are dry at harvest. Damage of the fruit coats could also occur, making them permeable to water. In the 'parenchyma cells' skinning mechanism this could be due to cells being damaged during drying (Lewicki & Pawlak, 2003), and in the 'cementing layer' skinning mechanism cracking of the cuticles and pericarp due to excessive exposure to moisture could occur (Knoche & Peschel, 2006; Aloni et al., 1998). Results

presented in chapter 5 shows that grains with partial loss of husk (missing lemma) germinate faster and develop higher levels of enzymes than grains with attached husk, or those without any husk. It is therefore possible that the mechanism of skinning as well as the type of husk-loss is important. Grains expressing the cementing layer mechanism of skinning, usually lose lemma or both lemma and palea, with no fragments of husk attached

Results from chapter 4 indicated that a large proportion of skinning in a sample resulted in high inhomogeneity and undermodification of the grains. This could be a result of faster water uptake into the grain followed by faster germination and the grain failing to germinate due to hypoxia. Barley grains are very well equipped to surviving lack of oxygen, even for prolonged periods (Wilhelmson et al., 2006), however hypoxia results in changes to metabolic processes of the grain, including induction of secondary dormancy (Hoang et al., 2013). Barley, unlike some other cereals, will not be able to complete germination in absence of oxygen (Loreti et al., 2002; Perata et al., 1998) which eventually results in embryo death (Perata et al., 1996). Equally the inhomogeneity of samples could be a result of slower uptake of the water into the grains due to potential damage of the embryo, and slower germination or complete failure to germinate. Brookes et al. (1976) and Swanston et al., (2006) has shown that water uptake in barley grain is dependent on the variety as well as the environment it has been grown in, with different varieties having varying susceptibility to the impact of environmental factors. This is in part a result of the structure of the endosperm: mealy structure has less protein and is floury, steely structure is much denser and does not modify evenly. Endosperm structure

has long been shown to impact on malt quality and steely grains do not hydrate and modify as well as mealy (Palmer, 1975b; Chandra et al., 1999; Koliatsou & Palmer, 2004; Ferrari et al., 2013; Holopainen et al., 2005).

Grains used in the experimental work in chapter 3 and then chapter 4 would have most likely expressed a mixture of two mechanisms of skinning. They were grown in the field and combine harvested, therefore my results indicate that skinning along the cementing layer would most likely have occurred. But the grains were also then threshed in the laboratory, after they have been dried, to create the severe skinning categories This most likely resulted in the 'parenchyma cells' skinning mechanism in addition to the 'cementing layer' skinning that has existed already. Chapter 5 begins to characterise the differences in type of husk-loss in samples from field, where mechanism of skinning is along cementing layer. The differences in germination rate of the grains with lemma missing and huskless highlights that type of husk-loss plays a significant role in modification of the grains. However these differences might not be significant in the large bulk of grains; future studies should attempt to quantify this importance on a larger scale than this thesis did. Additionally, impact of different skinning mechanisms on the quality of malt produced could be just as important as the type of husk-loss and requires further investigation. Future research should focus on investigating the influence of the skinning mechanism on the malt quality and progress modification. The ideal starting point would be experimental work carried out on skinned grains from one single variety grown in the glasshouse and in the field.

6.3 Malt quality in relation to skinning mechanism and husk-loss type

Modification of the malt and the homogeneity of this process have been shown in this thesis to depend not only on the severity of skinning but also on the type of husk-loss. This is another point which proves how important it is for skinning to be addressed and how skinning causes inhomogeneity in number of different ways. A novel finding in this thesis was that grains with missing lemma develop much higher levels of α-amylase and germinate faster, whereas huskless grains are the slowest to germinate and have the lowest levels of α-amylase. Although the exact mechanism of this is unknown, there are several factors that can improve or slow down α-amylase production. Examples of this include low oxygen availability, which slows down production of enzymes (Loreti et al., 2002), increased sugar concentration which blocks GA signalling (Perata et al., 1997) or increased sensitivity of the embryo to ABA (Benech-Arnold et al., 2006). Lemma missing is the most common type of husk-loss in the field sample; faster germination can lead to overmodification. In contrast those grains with the entirety of the husk missing were the slowest to germinate and develop hydrolytic enzymes. This further demonstrates that simple adjusting of the malting regime would be a sufficient solution for barleys with even husk-loss (Agu et al., 2008; Swanston & Middlefell-Williams, 2012). In addition to the homogeneity of the barley and malt, homogeneous skinning, including the type of

husk-loss and the mechanism of skinning would be easier to address than those mixed husk-loss types and mechanisms. This however is not feasible and reducing skinning is the best way forward to producing the highest quality malt.

Hot water extract has been used successfully to assess the quality of malt produced for a very long time. It is a quick and reliable method, and although the levels of sugars in HWE are much lower than those recovered from the same malt in brewery or distillery, the translation of experimental result to large scale performance is very well established and understood. However the continuous drive for improved extract from modern barley varieties could be leading to breeders unwillingly selecting for varieties with poorly adherent husk because samples with increased severity of skinning had an increased HWE. The values for HWE in the UK in 1939 were recorded around 288 l^o/kg, and have since then risen to around 308 l^o/kg (Briggs, 1998). In addition the yields of barely have increased from around 2.5 t/ha to 5 t/ha in years 1949-1983 (Silvey, 1986). Results presented in this thesis therefore clearly highlighted the inadequacies of HWE as a measure of malt quality, especially in bulks with even a small proportion of skinned grains. This is wider problem and inadequacies of standard methods recommended by Institute of Brewing have previously been shown (Palmer, 2000; Darlington & Palmer, 1996; Monnez & Flayeux Muu-erandM Moll, 2012; Briggs, 1998). The inflation of HWE in skinned samples is not unexpected as HWE uses 50 g of grains, and husks do not add value to the extract, but constitute between 10% to 17% of grain weight. This dilutes the sample, resulting in lower values of the extract recovered, similar observation was made by Swanston et al. (2017) and Meredith (1959). This inflation of HWE only

became apparent in the present study after adjusting the extract for the husk biomass lost, this however is not a routine adjustment used in the industry. The barley samples corrected for the husk-loss were previously described by Meredith (1959), however the researchers did not publish the full method of adjusting, only the final results were available. Adjustment method used in this thesis could be used as a starting point in the development of a calculation for samples with a proportion of skinned grains. This would give the industry a quick guide on malting regimes and better representation of the expected extracts. Such adjustment could however prove challenging to develop, as different varieties have varying thickness of the husk and constitute 10% to 17% of the grain weight (Evers et al., 1999). Grains of the same variety grown in different environments also have different malting qualities (Swanston et al., 2006) and skinning varies between environments (Aidun et al., 1990). There are a lot of factors influencing grain size and development, and this could be limiting in how the adjustment for skinning could be developed.

Plant breeders developing new varieties need to be especially aware of skinning as the pressure to produce varieties with reduced dormancy and increased extract values could cause the breeders to inadvertently breed in the genes responsible for weak husk adhesion. Removing husks alleviates dormancy (Lenoir et al., 1986; Dunwell, 1981), making the variety more desirable and husks do not add any value to the extract. The tests used in evaluation of malt and varieties relay on a weight of a sample. Samples with missing husk will have higher extract levels, as the proportion of the starchy endosperm in such sample is higher than in intact grains. This was evidenced in chapter 3; samples with large proportion of skinned grains had

high HWE. It was only after correcting this for the weight of the husk biomass lost and using the number of grains, rather than standard weight, it was clear that the HWE achieved was much lower. If this adjustment was not performed, and skinning was not accounted for in the quality testing, samples with high skinning would have the highest extract. Process of selecting new varieties into the AHDB Recommended List evaluates the agronomic performance, the yield and malting performance of the new varieties. It is a strict evaluation process, with statistical methods employed. However the final decision on adding or discarding new varieties that are very similar in their performance can be done solely on yield and HWE. The difference between those varieties that make it onto the recommended list and those that are rejected can often be just 1 L°/kg, with little consideration to other characteristics.

The above arguments reiterate the importance of addressing skinning at the point of breeding and when developing new varieties. There is a large scope for skinning to cause inhomogeneity and losses along the whole supply chain. Breeding new varieties for higher extract could be the direct cause of the increase in skinning severities in new varieties.

6.4 Novelty of the research and contributions to scientific knowledge

This thesis presents several novel contributions to the understanding of the mechanism of skinning and the effect this condition has on the efficiency of malt

production. Experimental work in chapter 2 has shown that two skinning mechanisms are present, occurring based on the moisture content of the barley grains and potentially hydration levels of individual tissues. Dry grains skin through 'parenchyma cells' mechanism and short exposures to moisture in dry grains protect from skinning, long exposures and high moisture content results in 'cementing layer' skinning mechanism, in which husk adhesion is weak. The impact of moisture on the adhesion could be further studied and developed into formal guidelines for farmers, offering advice on the best conditions for the crop to be harvested to minimise the severity of skinning. It is also clear that these contrasting effects of moisture on the grains are related to two different types of skinning. Previously 'cementing layer' skinning mechanism was described by Gaines et al. (1985) and Hoad et al. (2016) and 'parenchyma cells' mechanism of skinning was described by Olkku et al. (2005). However this thesis was the first to show that the two mechanisms of skinning occur depending on the environment and MC of the grains. In the glasshouse grown grain, harvested and threshed at very low moisture content, breakage of parenchyma cells is the most prominent skinning mechanism. In the field the failure of cementing layer was most noticeable, supporting the view that it is the composition of this layer, which is responsible for adhesion and environmental factors could alter it (Brennan et al., 2017a, 2019).

Evidence of the influence of skinning on malting has mostly been anecdotal and historical. This thesis presents a clear link between the quality of barley grain and the quality of malt produced, and quantifies the effects of skinning on the homogeneity of malt. This problem is increasingly present in modern barley varieties

(Brennan et al., 2017b), and it is possible that breeders, striving for the highest extract possible, inevitably breed this condition into the new varieties. Research in this thesis has proven that barley with large proportion of skinned grains produces higher HWE, and only after an adjustment of the calculation, it was clear that this was due to lost husk biomass. Large proportion of skinned grains resulted in undermodification of the malt, and in reality loss of extract, which could have been overlooked if only conventional methods of analysis were employed, or if a very small proportion of skinning was present.

Finally, it is not just the presence or absence of the husk, which influences the progress of modification and germination in individual grains. The evidence from experimental work in this thesis suggests that partial loss of husk (lost lemma) germinates and modifies much faster than grains with fully attached or fully missing husk. According to the data in this thesis lemma missing grains cause overmodification of the malt. Future research should compare malts produced from grains with different husk-loss types, to determine whether the results presented in this thesis are replicated during micromalting. This could also be linked with the different skinning mechanisms to better understand its role in efficient malt production.

6.5 Limitations of the research and future studies

The experimental work here used very few of the available malting varieties. However, the results of this thesis and those of Brennan et al. (2017b) both indicate that the difference in skinning severities in modern varieties are not significantly different from each other. This poses a challenge in breeding in itself: finding material of suitable quality is very difficult, when yield and extract are the most important factors. Those modern barleys could be compared with more traditional varieties, which have remained a favourite for many brewers. There is a growing trend, especially amongst craft brewers to use some of the older malting barley, like Maris Otter or Golden Promise, due to its unique flavour characteristics as well as good performance during malting and brewing, one of those performance characteristics being very low skinning (MAGB, 2019).

This thesis was the first to describe two separate skinning mechanisms, dependent on grain moisture at harvest. Investigation into the effect of each of the skinning mechanisms on the malting process, and whether these two skinning mechanisms differed from each other in their influence on modification and malting efficiency should become the focus of future studies. It is possible that one mechanism has a different effect on the germination and the rate of enzyme production of the grains than the other. Answering this question would provide understanding of one more component of malt homogeneity.

It was evident from the experimental work that type of husk-loss in the grin bulk could also contribute to producing inhomogeneous malt. This should be investigated on a larger scale as well as in connection with water uptake, as the results on water uptake presented in this work could not be conclusively linked to germination and α-amylase production. Interaction of the skinning mechanism and husk-loss type is also a possible and should be taken into consideration in any future experimental work.

Finally, future studies could be beneficial to the industry and farmers. Clear guidelines on the best conditions for harvest could be developed to aid farmers in their decision making. A universal adjustment method could be developed for maltsters, which would allow for easy correction of the samples with skinning problems. Breeders should also look into in more detail into the grains their using in their programs, and make sure they are not breeding skinning into the new varieties.

6.6 Importance of grain skinning

Quality of harvested barley grains has a direct impact on the quality of malt produced, and skinning is one of the conditions which affects the efficiency of malt production through the whole supply chain. Homogeneity and efficiency are the two aspects appearing throughout this thesis to describe the negative impact of skinning on malt. Developing new varieties with low skinning susceptibility would improve the homogeneity and efficiency. This would be beneficial to the famers and maltsters, both in terms of improved prices of barley for the farmers and improved malting

process and the end product for maltsters. Improvements in homogeneity of barley and malt and in the efficiency of the processes were the two main areas, which this work was concerned with.

In chapter 2 experiments aimed at investigating the influence of moisture on skinning, which would result in harvest of more homogeneous sample. Chapter 3 quantified the effect of skinning on malt, followed by detail, single grain investigation of modification in chapter 4 and 5. These two chapters looked at how skinning causes inhomogeneity in malt, whether undermodification or overmodification is responsible for the lack of efficiency and how exactly the type of skinning influences the germination and modification of the grains. Loss of husk in barley is preventable and measures such as variety choice, settings of the combine harvesters and as evidenced in this thesis and other publications conditions during harvest have an impact on severity of skinning. Improvements in quality of harvested grains and in efficiency of malting, would lead to increased productivity and efficiency of the crop use. The agricultural land in UK is used up to its maximum potential, and covers in 2008 it approximately 77% of total UK land area was used for agriculture, therefore growing larger quantities of crops is not a viable option (Angus et al., 2009). The only solution is to make the most of the plants we are already growing. The demand for malt is still on the increase, with Scotch whisky being one of the highest exports in Scotland and craft brewing sector still experiencing growth. Efficiency of the whole system would ensure that the demand of brewing and distilling sectors is met, without wasting the valuable resources including water, energy or land during growing, malting or brewing and distilling the grains. UK maltsters buy 2 M t of barley

each year, from which 1.5 M t malt is produced (MAGB, 2019). In Scotland alone average turnover for distilling industry was 5.3 million in 2018 (O'connor, 2018), a 1% loss in production has significant impact on the whole of malting, brewing and distilling sectors, therefore improving grain quality is essential to secure the most efficient malting.

6.7 Conclusions

Skinning is a detrimental condition of malting barley grain, of increasing importance for the industry. It influences the homogeneity of the final malt, samples with large proportion of skinned grains do not modify fully and the potential extract is not fully recovered. This thesis has achieved novel findings in understanding skinning and its influence on malting process. Moisture content is of great importance during growing season and harvest. Dry grains are susceptible to husk-loss, whereas moisture associated with earlier growth stage protects grains from skinning. In contrast excessive moisture at harvest, like that caused by rainfall exacerbates skinning. Samples with large proportion of skinned grains produce undermodified malt, resulting in less extract. The grains do not fully modify when the embryos or growing acrospires become damaged. This stops the development and release of hydrolytic enzymes in the aleurone layer and the breakdown of cell walls in the endosperm also stop. Methods commonly used to measure varietal differences in the quality of malt are not adapted to account for samples which have lost their husk. One such measure

is HWE, which is higher in skinned grains, but when adjusted for the husk biomass lost it was much lower than intact grains.

This thesis has produced novel research on causes and effects of skinning and quantified this problem. Based on the results from this work it would be recommended for the farmers not to harvest grains after a heavy rainfall or after a hot and dry spell, as this could result in increase of the skinning severity. Maltsters and breeders would be recommended to account for skinning when evaluating new varieties or bulks of grain at intake. Breeders especially should avoid using varieties with high skinning severity as a source for developing new varieties. Maltsters should set clear guidelines on acceptable skinning levels and account for this when malting their samples. There is a potential for this work to open up communication between farmers and maltsters in finding ways to improve barley and reduce skinning in grain. This would be beneficial to both industries and result in overall improved productivity form barley crops, without any need for increased input into growing this crop. The most important research question arising from this work would be to quantify the effects of the two different mechanism of skinning on the modification of barley. This should be related to the water uptake, germination rate and production of enzymes as well as the relation of the skinning mechanism with the husk-loss type. Answering this question would help to further improve malting efficiency and explain the role of skinning mechanism and husk-loss type in malt inhomogeneity.

Chapter 7. References

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